Impacts of selecting sheep for resistance and resilience to gastro-intestinal nematode parasites

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Abstract

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This study investigated the impacts of decades of divergent selection for either resistance or resilience to gastrointestinal nematode (GIN) parasite infection in Romney sheep lines and involved a series of three studies.

Study 1 (Chapter 3), assessed the variation in timing of development of immunity and physiological maturity in Romney selection lines; one selected for resistance and the other selected for resilience when exposed to natural mixed-species GIN parasite infection with no anthelmintic treatment. From weaning at mean 92 days-of-age, animals (n=53) were sampled for faecal egg count (FEC) expressed as eggs per gram of faeces (epg), saliva for immunoglobulin (IgG and IgA) determination and fasted live weight (LW) every 10 days until 351 days-of-age. Overall, mean back-transformed FEC were consistently low for resistant animals (<200 epg) whereas resilient counterparts' FEC increased with time to reach a peak of 1400 epg at day 230 for females and 1800 epg for males at day 280 before declining to less than 500 epg by day 300, respectively (P<0.001). Immunoglobulin for *T. colubriformis* L3-specific IgG responses showed a selection-line by time interaction (P<0.001) reflecting an earlier rise in IgG profiles in resistant compared with resilient line but both reached similar magnitudes (1.53 ± 0.16 OD) before declining from day 330. Resistant lambs reached the threshold IgG of 1-OD indicative of presence of immunity earlier at 220.6 ± 8.8 days-of-age compared with resilient-line animals which reach this threshold 40 days later at 263.4 ± 6.9 days-of-age, respectively (P<0.001). In addition, resistant females were also associated with an earlier indication of sexual maturity depicted by the presence of mating marks by a vasectomised ram compared with their resilient counterparts *viz.* 263.5 ± 3.7 *c.f.* 274.4 ± 3.4 days-of-age, respectively, (P=0.048). Mean fasted live weight showed a selection line by time interaction (P<0.001) which reflected greater LW in the early phase of the study in resilient males but increasing for all groups until day 280 before declining and being similar for all groups from day 330. Correspondingly, greater proportions of

resilient male lambs (81.3 %) reached the arbitrary slaughter weight of 35 kg by day 215 whereas only 30% of resistant male lambs reached the mark and only 35.7% and 23.1% of resilient and resistant female lambs reached the slaughter weight, respectively. There was a temporal difference in the timing of immunity; resistant lambs developed immunity earlier, but even resilient-line animals developed immunity, although this was delayed. These findings allow for the suggestion that selection for resistance to parasites may have favoured animals with a lighter body weight that develop immunity at a younger chronological age. However, at the same chronological age, resistant animals appear to be physiologically more mature compared with resilient-line animals. Further there was evidence that resilient Romney lambs still retain the capacity to develop an effective immune response to parasite challenge but this takes longer which may explain the higher indication of infection in these animals. This difference in the timing of immune development has implications for breeding programmes as to when to impose selection pressure for resistance to nematode infections without compromising protection. However, the greater growth potential of resilient lambs earlier in their lifetime inspite of increasing levels of parasite infection, provides a short-term opportunity for commercial sheep producers to exploit and target higher premium prices that are often scheduled early in the season but in the long-term this option may pose greater risks for pasture contamination. It was also apparent that a combined selection for growth and immunity can be achieved and would require use of weaning weights of >23 kg followed by sampling for either immunoglobulin G around 220 days-of age or FEC around 270 days-of-age.

The second study (Chapter 4) examined the epidemiological advantage of resistance to parasite infection and whether it outweighs the opportunity costs of greater growth potential in resilient Romney selection-line animals that was evident in study 1. Resistant and resilient line animals were maintained in farmlets from birth through until 7-months-of-age. The study involved 4 farmlet-based treaments *viz.* resistant alone with no drench (RtA), resilient alone with no drench (RlA), resistant and resilient mixed drenched (Mxd+Dr) and resistant and resilient mixed with no drench (Mxd). Lambs were born and remained set-stocked in their respective birth paddocks within each farmlet throughout the study period and sampled monthly for FEC, saliva, live weight, herbage mass (kgDM/ha) and number of infective larvae per kilogram dry matter of herbage (L3 per kgDM) from weaning until around 30 weeks-of-age. Back-transformed FEC (epg) tended to show an interaction between treatment and time (P=0.058) reflecting greater FEC in RlA and Rl+Mxd groups which also increased with time to reach a peak of 800 and 500 epg, respectively, whereas FEC for RtA, Rt+Mxd and Rt+Mxd+Dr groups were consistently low. For *Nematodirus* Spp. FEC, there was a tendency for an interaction between treatment and time (P=0.090) reflecting a decline in FEC with time in RlA and Rl+Mxdr+Dr lambs whereas in Rl+Mxd group FEC rose on day 180. *Strongyle* pasture larvae were greatest in RlA and Rl+Mxd lambs *viz.* 952.0 and 897.7 L3 per kgDM compared with resistant alone

(140.4 L3 per kgDM) and mixed drenched groups (78.2 L3 per kgDM) respectively (p=0.028) which increased with time to reach a peak of 2757.5 and 1801.1 L3 per kgDM in RlA and Rl+Mxd, respectively, whereas in RtA and Mxd+Dr groups remained low (P<0.05). There was no interaction between treatment and time (P=0.477). Pasture larvae for *Nematodirus* spp. showed an interaction between treatment and time (P<0.007) reflecting a rise in larval numbers in farmlets grazed by RlA and Rl+Mxd lambs with two peaks one on 150 *viz.* 5700 *c.f.* 2700 L3 per kgDM and the other on day 210 *viz.* 8700 *c.f.* 3300 L3 per kg DM despite a declining FEC whereas RtA and Mxd+Dr farmlets had typically less than 300 L3 per kgDM. Overall, cumulative liveweight gain (LWG) was greater in Rl+Mxd+Dr group (19.48 ± 0.91 kg) compared with Rt+Mxd+Dr treatment group (17.22 ± 0.78 kg) respectively (P<0.05) and was similar between Rt+Mxd and Rl+Mxd groups *viz.* 17.16 ± 0.84 kg *c.f.* 16.99 ± 0.84 kg (P>0.05). Further LWG was also similar between RlA and RtA *viz.* 14.42 ± 0.45 kg *c.f*. 13.85 ± 0.48 kg, respectively, (P>0.05) although this was temporary; being greater in RlA compared with RtA in first 60 days post-weaning but was considerably reduced in the last 50 days in RlA than in RtA representing losses of productive potential of 28.9% and 16.3%. Overall, these results provide further support that selection for resistance does confer epidemiological benefits in reducing larvae contamination on pasture, a consequence of lower worm egg excretion. However, there was no evidence from the two years study to suggest that the considerable epidemiological benefit provides the added advantage for greater growth in resistant animals when compared with resilient under challenge with no anthelmintic treatment, at least in the short term, which suggested that the epidemiological advantage of resistance over the time frame of this study equalled the costs.

Consistent with the findings of Chapter 3 (study 1) and Chapter 4 (study 2), Chapter 5 (study 3) investigated faecal ('parasite') avoidance grazing behaviour between Romney selection lines. The study was designed to answer whether the differences in parasite loading between selection lines animals could be attributable solely to immunity or whether they may also reflect differences in grazing behaviour. Ten lambs from each selection line that were maintained in separate farmlets and naturally exposed to mixed-species nematode parasites were assessed on distance grazed from either a mud-clay or faecal ball at 130, 150, 180 and 210 days-of-age. Resistant-line animals maintained lower faecal-egg counts compared with their resilient counterparts *viz.* 25.6 *c.f.* 771.0 eggs per gram of faeces (epg), respectively, (P<0.001). Mean *Trichostrongylus colubriformis* L3-specific immunoglobulin IgG was greater in resistant than in resilient animals (P=0.005) and increased with time (P<0.001) but there was no interaction between selection line and time (P=0.130). Overall, grazing distance from the object was greatest for faecal ball, *viz*. 11.53 ± 1.14 cm compared with mud-clay ball, *viz*. 4.19 ± 1.00 cm (P<0.001) and decreased with time (P<0.001). Grazing distance tended to be greater for resilient, *viz*. 8.63 ± 1.14 cm than resistant, *viz*. 7.09 ± 0.99 cm (P=0.09) but there was no interaction between selection line and ball type (P=0.709) indicating similar levels of

faecal-specific aversion between the selection lines. These results suggest that the lesser infection levels in resistant-line animals when grazing is unlikely to be due to reduced exposure to infective larvae as a consequence of greater faecal avoidance.

In summary, selection has resulted in animals with divergence in their response to gastrointestinal parasites. However, these studies suggest that common fundamental characteristics still exist, with a magnitude of the difference reflecting the timing of the response to infection. This timing when applied to a field context does result in considerable epidemiological differences which can be attributed to the earlier, but not necessarily more powerful immune response and cannot be attributed to differences in grazing behaviour/ faecal avoidance. The implication of this is that either approach in its entirety has similar outcomes in animal production, however, in combination with anthelmintic the greater growth potential of resilient animals is beneficial. Further, there is some evidence of sexual dimorphism indicating that selection in females may not create the same production difference a factor which could be exploited in selection programmes..

Keywords: Romney sheep, selection, resistance or resilience, gastro-intetinal nematode parasites, infection, epidemiology, impact, immunity, physiological development, nutrition, animal productivity, faecal/ parasite avoidance, sexual dimorphism.

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Publications during the course of the study

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Greer, A. W., **Hamie, J. C**. and McAnulty, R. (2016). Resistance vs. resilience: A solvable dilemma? *Proceedings of the Society of Sheep and Beef Cattle Veterinarians of the NZVA Conference*, pp, 65-71.

Yan, J, Dukkipati VSR, Blair, HT, Biggs PJ, **Hamie, JC,** Greer AW (2017). A genome-wide scan of positive selection signature using INFINiUM® HD SNP Beadchip in two Romney lines, selected for resistance or resilience to nematodes. Proceedings of the Association for the Advancement of Animal Breeding Genetics, 22: 585-588

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

Gastro-intestinal nematode (GIN) parasites are a major disease constraint to ruminant production (Fabiyi, 1987; Over et al., 1992; Perry et al., 2002). They cause substantial production losses in grazing ruminants, most specifically young animals and older animals in the periperturient period (Sykes, 1994). Producers incur costs associated with both loss in animal productivity and control of the disease (McLeod, 1995; Nieuwhof & Bishop, 2005; Sackett et al., 2006), which relies mainly on administration of anthelmintics (Besier & Love, 2003; Woolaston & Baker, 1996). The wides-pread development of anthelmintic resistance by parasite genera to most broad-spectrum deworming drugs (Dobson et al., 1996; Hughes et al., 2007; Le Jambre et al., 1999; Leathwick et al., 2009; Leathwick et al., 2001; Pomroy, 2006; Waghorn et al., 2006) renders this control strategy unsustainable. There is a need for integration of a number of complementary measures for effective GIN control, involving approaches that incorporate parasite biology and measures that shape sheep farming systems and management decisions.

Selection for nematode resistance in the host is a gradual but arguably more sustainable approach to the control of the disease. Selection of sheep with enhanced resistance to GIN parasites or resilient animals that require minimal drenching regimes (Bisset et al., 2001; Bisset et al., 1996a), is a control measure that complements other strategies (Bishop et al., 2004) and may be used as a long-term control measure (Bishop & Stear, 1997, 1999). Resistance to infection is here defined as the animal's ability to invoke and sustain sufficient levels of immunological responses when exposed to larval challenge to suppress parasite establishment and subsequent development and / or eliminate worm burdens (Bisset et al., 2001; Woolaston & Baker, 1996). Contrary "resistance to the effects of infection" termed "resilience" (Riffkin & Dobson, 1979) or "productivity under challenge" (McEwan et al., 1997) is the animal's ability to withstand prolonged effects of nematode parasite challenge with minimal anthelmintic treatment while maintaining acceptable levels of productivity (Morris et al., 2010; Woolaston & Baker, 1996). Some breeds, and individuals within a breed, are known to be more resistant to GIN than others (Baker & Gray, 2004; Gray, 1991; Gruner & Cabaret, 1988) and resistant animals harbour fewer parasites than susceptible ones (Bisset et al., 1991c; Miller et al., 1998; Morris et al., 1995b; Woolaston & Piper, 1996). The acquisition and expression of immunity (resistance) against nematode parasites is genetically controlled (McManus et al., 2014; Windon, 1996; Woolaston et al., 1995a) with 85% of the genetic variation occurring within flocks (Woolaston & Eady, 1995). Generally, indigenous breeds that have evolved under continued strong parasite challenge are known to be more resistant to *Haemonchus contortus* compared with less adapted

European breeds under the same conditions (Amarante et al., 1999a; Baker, 1995; Baker & Gray, 2004; Baker et al., 2003a; Bishop & Morris, 2007; Courtney et al., 1985; Gamble & Zajac, 1992; Gray, 1991; Matika et al., 2003; Mugambi et al., 1997; Preston & Allonby, 1979). Animals from resistant lines are often observed to have reduced productivity in temperate countries, such as New Zealand (Baker & Gray, 2004; Woolaston & Baker, 1996) and combined with other undesirable characteristics, such as breech soiling and associated susceptibility to flystrike, make them unfavourable in commercial farming conditions. Even in divergently selected Romney and Perendale sheep lines, reports appear to suggest that resistant animals tended to be smaller compared with resilient lambs when both are grazed together under challenge (Morris et al., 1996; Morris et al., 2000; Morris et al., 2005). de Lautour and de Lautour (2010) reported that the top ranking 60 resistant rams had an average 6.55 kg lower breeding value for live weight at eight months of age compared with resilient counterparts that were selected from the same base flock. Bisset et al. (2001) thus stated that few farmers would be satisfied with animals that can withstand high parasite challenge but with no demonstrable ability for increased productivity.

In New Zealand, lines of Romney sheep selected for their resistance, susceptibility and or resilience (or production under challenge) have been established and maintained since 1979 and 1994, respectively, (Bisset et al., 1996a; Bisset et al., 1994; Morris et al., 2010). Selection for resistance has made substantial achievement in reducing worm faecal egg count (FEC) in a resistant Romney line (Bisset et al., 1991b; Morris et al., 2000; Morris et al., 1995b; Morris et al., 2005) whereas selection for resilience has increased the average age-at-first drench by 24 days relative to the unselected control animals (Bisset et al., 1996a; Morris et al., 2010). Surprisingly however, there remains a dearth of information on the epidemiolgical consequences of either of these approaches or the biology that controls the timing of immune dvelopment. Much of the selection in these Romney lines has been based on phenotypic marker trait (FEC) which has been argued as being an imperfect measure of resistance (Greer & Sykes, 2012; McRae et al., 2015; Sweeney et al., 2016). Earlier, Bishop and Stear (1999) observed that there was no intuitive relationship between productivity and resistance to enable developing genetic selection indices for both traits. Although preliminary selection indices of combining FEC and production traits began in 1994, Morris et al. (1995b) suggested that information was still lacking on the way to most appropriate combine resistance and production traits. At least in part, acquisition of immunty is known to be nutritionally costly to the host animal (Greer, 2008) as it is associated with the comensuration of a threshold of immunoglobulins responses which are mostly proteinaceous in nature.

Previous studies investigating the production differences between lines of animals selected for resistance or resilience have reported greater levels of productivity in resilient animals (Morris et al., 2000; Morris et al., 1997; Watson et al., 1992; Woolaston, 1992). However, these comparisons have

always been made where lambs of each phenotype are grazed together resulting in potential masking of the epidemiological benefits for the resistant animals due to greater levels of worm egg deposition from resilient counterparts and resulting lavae. This may have resulted in underestimation of the impact of host genotype on FEC and live weight (Laurenson et al., 2012). This project therefore sought to evaluate the longer term impacts of either resistance or resilience to GIN on epidemiological characteristics such as, population dynamics and performance of Romney selection lines grazing separately on farmlets where the epidemiological impacts of either reduced (in the case of resistant animals) or greater (in the case of resilient animals) pasture contamination can be determined and/or controlled. The study further investigated variations in the timing of immunological antibody responses and whether differences in the grazing behaviour exist that would account for the commonly observed difference in parasite loading between Romney selection lines.

Chapter 2

Literature Review

2.1 Introduction

Gastrointestinal nematode (GIN) parasites are a major disease constraint to sheep production, resulting in substantial production and economic losses to the sheep industry. Control of GIN has relied on administration of anthelmintic drugs (Besier & Love, 2003; Leathwick et al., 2009; Leathwick et al., 2001; Pomroy, 2006; Woolaston & Baker, 1996) but the development of widespread anthelmintic resistance renders this control strategy unsustainable. Selection of animals that are genetically either resistant or resilient to nematode parasite are two options that are often debated but widely used tools in the search for sustainable parasite control strategies.

This literature review, provides a background of existing knowledge epidemiology, factors the influence immune development, selection for resistance or resilience, prediction models and actual impacts of selection and ends with summary.

2.2 Parasites, epidemiological, impacts of interaction and grazing behaviour

2.2.1 Parasites and their life cycle

Parasites are living organisms that live on or within a host, and derive their sustainance at the expense of the host. There are numerous kinds of GIN parasites, but only a few are regarded as economically important in ruminant animals which belong to the order *Strongylida* and are given in Table 2.1. Nematode prasites such as *Haemonchus contortus*, *Teladorsagia circumcincta*, and other *Teladorsagia* spp. and *Trichostrongylus axei* and the small intestinal species of *Trichostrongylus colubriformis* and other related species*, Nematodirus* and to a lesser extent *Cooperia* (Vlassoff et al., 2001) are among the economically important species in ruminant animals. A compilation of the morphological identification characteristics of the economically important nematode parasites are detailed in Roeber et al. (2013). The occurence and relative abundance and/ or prevalence depends on geographical region and associated climatic variables that favour their development and survival (Barger, 1999; Morgan & van Dijk, 2012) as well as species of livestock kept and factors that influence farm management decisions.

Table 2:1 Nematode parasites of sheep in New Zealand (compiled by Vlassoff et al., 2001)

Lung	
Dictyocaulus filarial	*Muellerius capilaris*
Protostrongylus rufescenes	
Abomasum	
Haemonchus contortus	*Teladorsagia circumcincta*
Teladorsagia trifulcata	*Teladorsagia pinnata*
Teladorsagia crimensis	*Teladorsagia ostertagi*
Trichostrongylus axei	
Small Intestine	
Bunostomum trigonocephalum	*Capillaria bovis*
Cooperia curticei	*Cooperia mcmasteri*
Cooperia oncophora	*Cooperia punctate*
Nematodirus abnormalis	*Nematodirus filicollis*
Nematodirus furcatus	*Nematodirus helvetianus*
Nematodirus spathiger	*Strongyloides papilosus*
Trichostrongylus capricola	*Trichostrongylus colubriformi*
Trichostrongylus vitrinusn	
Large intestine	
Charbetia ovina	*Trichuris ovis*
Oesophagotomum columbianum	*Oesophagotomum venulosum*

2.2.2 Life cycle

Most GIN parasites have a similar direct life cycle that does not requires an intermediate host (Familton & McAnulty, 1997). Adult worms in the gastrointestinal tract mate and females produce eggs (ranges 70-150 µm in size), the number depends on the species involved. For example, *Haemonchus* females produce about 5,000-10,00 eggs per day whereas *Teradosargia* and *Trichostrongylus* produce between 100-1000 eggs per day. Eggs are excreted in the faeces of grazing animals. When weather conditions are conducive for development, eggs hatch into first-stage larvae (L1) which then moult two times into the second-stage (L2) and then the infective third-stage (L3) that migrate out of the faeces and into the herbage where they may be ingested by grazing ruminants. Except for *Nematodirus* species whose larvae develops inside the egg and hatches at infective larvae (L3) stage. Following ingestion by a suitable host, the L3 larvae ex-sheath to L4 in response to changes in CO_2 concentration, temperature and pH before they reach their site of infection (Vlassoff et al., 2001).They then undergo two further moults to complete the development

to reproductive adults, which takes about 15-21 days from ingestion as L3 for most common species (Familton & McAnulty, 1997). A summary is given in Figure 2.1

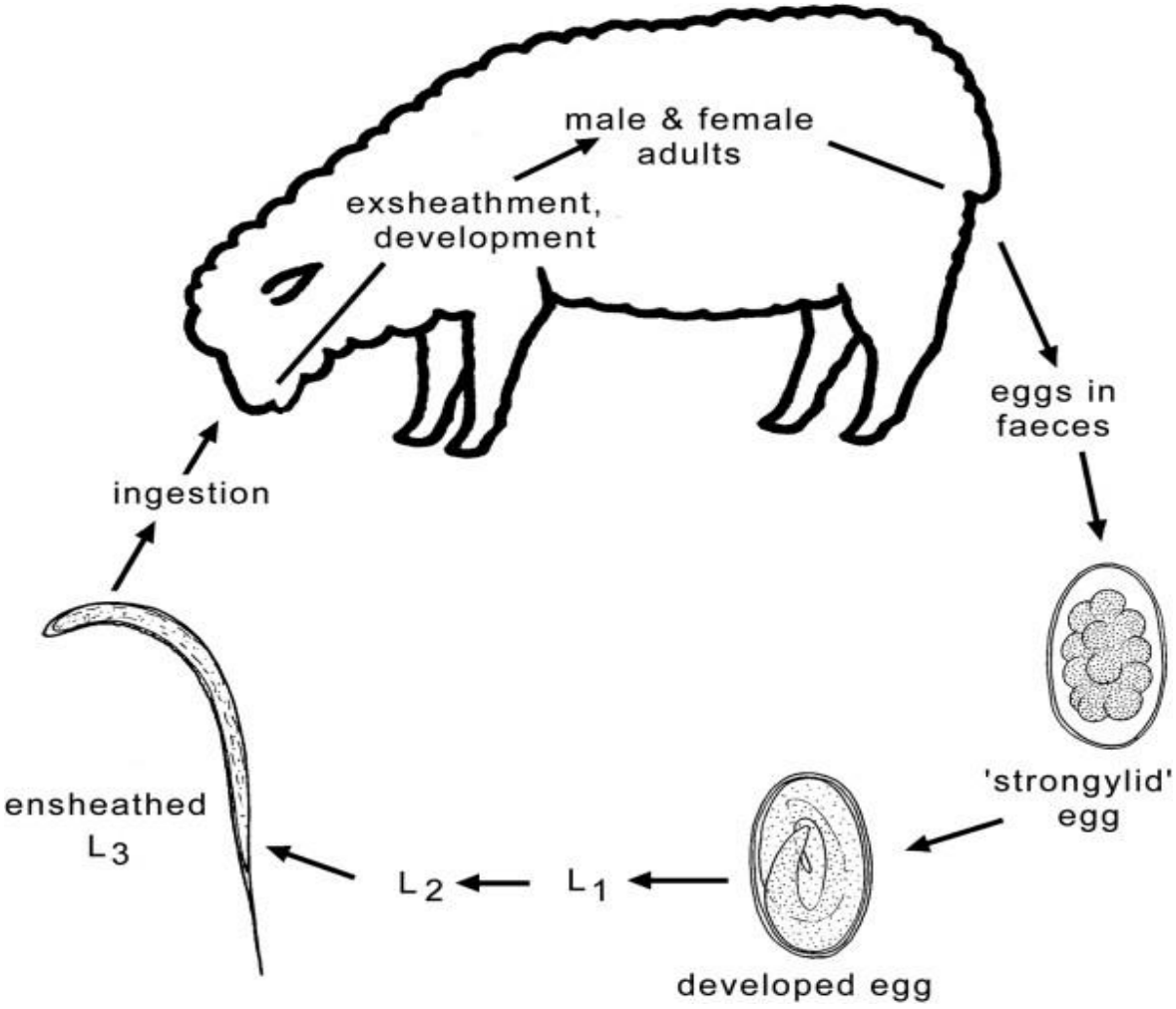


Figure 2:1 Summary of nematode life cycle (Source: Roeber et al 2013)

2.2.3 Epidemiology

Epidemiology is hereby defined as the interplay of various factors that determine the populations dynamics of nematode parasites in the host animals, their free-living state as well as their geographical distribution. This knowledge is vital in understanding the sources of peak larvae availability on pasture, as factors that govern their seasonal patterns (Barger, 1999) could help in identifying the potential risks of disease outbreaks and in devising control management decisions.

In New Zealand, the epidemiology of sheep nematode parasites has been comprehensively reviewed Vlassoff et al. (2001). Typically, the numbers of nematode parasite eggs start at low levels in early spring, then rise to reach peak in mid-summer to early autum, before declining (Brunsdon, 1967, 1970; Stear et al., 1999; Vlassoff et al., 2001). Once eggs hatch and the resulting larvae develops to the infective stage, they can migrate vertically and laterally within habitats i.e. from faeces to surounding herbage or from faeces to soil and then back to herbage (Krecek & Murrell, 1988;

Leathwick et al., 2011; Waghorn et al., 2011) influenced by their ecological requirement for survival prior to ingestion. It is this complex spatial dynamic of larvae populations that make both predictions of levels of larval availability based on season or weather conditions, and quantification of larvae on pasture more difficult (Litherland & Deighton, 2008). Temperature and moisture are, however, the dominant weather variables that influence the success of development and survival of the free-living stages of parasites while pasture conditions play a modulating role (Levine & Todd Jr, 1975; O'Connor et al., 2006).

Temperature

Temperature has a major influence on nematode egg hatching, larval development and subsequent survival (Vlassoff, 1982). Both excess heat and extreme cold temperature are detrimental to larval survival. Variations do exists between paraite species in their optimum temperature range for development, but generally fall within the ranges of 15-35^{o}C for most nematode parasites. For example, *H. contortus* is reported to have the highest optimum temperature (25-37^{o}C) while *T. circumcincta* have the lowest optimun (25-30^{o}C) with the other species in between (O'Connor et al., 2006). With exception of *H. contortus*, eggs and larvae of most GIN paraite species may tolerate cold temperatures, as their metabolic rate is reduced (Familton & McAnulty, 1997) but very few develop into infective larvae (L3) stage. Specifically, eggs of *Nematodirus* spp. require prolonged exposure to chilling followed by a rise in temperature within 11-17^{o}C before hatching can occur (van Dijk & Morgan, 2008). But at temperatures below the maximum threshold, both egg and larval stage development rates increase with increasing temperatures (Morgan & van Dijk, 2012; Van Dijk et al., 2010). Salih and Grainger (1982) reported the time taken until hatching for *T. circumcincta* was shortest at mean temparature of 25^{o}C (1 day) and longest at 5^{o}C (11.8 days). Generally, eggs that do not hatch within a week or two of deposition fail to develop further, particularly at low temperatures (McKenna, 1998; Pandey et al., 1993; Waghorn et al., 2011). Ensheathed larvae are known to be more resistant and can survival longer in cold conditions (O'Connor et al., 2006; Stromberg, 1997) but their survival under tropical conditions is shorter (Cheah & Rajamanickam, 1997) owing to the warmer temperatures which increase metabolic rate and that rapidly depletes the energy reserves of the non-feeding L3 stage.

Moisture

Moisture is essential for parasite egg development, hatching, larval survival and subsequent migration onto pasture. However, there is no straight-forward relationship between moisture availability in the microclimate niches where larvae occupies and rainfall (Morgan & van Dijk, 2012). This is partly because development of both eggs and larvae in faeces do not depend only on rainflall but on other factors such as rate of evaporation, soil moisture and transpiration/hydration status of herbage (O'Connor et al., 2006). However, a thin film layer of water does favour development of

both eggs and larvae (Vlassoff, 1982) with optimal development occurring when faecal moisture content is in the range of 57-68% whereas at more than (>) 85% or less than(<) 55% moisture only <1% development may occur (Rossanigo & Gruner, 1995). Too high moisture content reduces oxygen concentration and delays development (Rossanigo & Gruner, 1995; Silverman & Campbell, 1959) and too little may lead to desiccation. For example in semi-arid regions, the absence of rain during drier months may bring parasite transmission to a halt (Agyei, 1997; Chiejina & Fakae, 1989; Onyali et al., 1990; Sissay et al., 2007a, 2007b). Generally, studies have reported a positive correlation between moisture and emergence of *Strongyle* larvae on herbage and / or the corresponding increase of worm burdens in tracer animals (Bryan & Kerr, 1989; Stromberg, 1997; Williams & Bilkovich, 1973).

Infective larvae of most GIN parasites develop in faeces and then migrate out of the faecal pat onto the surrounding herbage so that they may be ingested by the grazing animals (Stromberg, 1997). Larvae movement depends on the presence of a film of moisture on herbage (Bryan & Kerr, 1989; Lancaster & Hong, 1987; Niven et al., 2002; O'Connor et al., 2006) whereas absence of moisture can prevent emergence and migration of L3 out of faeces. The migration of L3 larvae from faeces has also been found to occur in waves which coincided with the presence of water and rainfall (Familton & McAnulty, 1997; Gronvold, 1989; Vlassoff, 1982). Larvae can move within 30 cm distance in a horizontal direction from the faecal mass and their concentration decreases with increasing distance from the faeces (Gronvold, 1989). Similar observations by Almeida et al. (2005) have reported that 89% of L3 were within 15 cm while only 11% were found between 15 and 30 cm from the faecal pat. However, greater distances of passive larvae migration (about 90 cm) may be acheived through rain drop splashes. Variations in concentration of larvae within herbage plant parts exist influenced by plant species and pattern of herbage growth (Morgan et al., 2007). The microclimate at the base of the herbage favours larval survival (Crofton, 1948; Levine & Todd Jr, 1975; Silangwa & Todd, 1964) and greater number of larvae have been reported within 25 mm of the grass stems with only 2-3% of larvae on the upper herbage (Crofton, 1948; Leathwick et al., 2011; Moss & Vlassoff, 1993; Rees, 1950; Silangwa & Todd, 1964) reflecting the high humidity and moist environment in those niches. Together these studies point to the importance of moisture (i.e. rain, irrigation water or dew) in determining where the greatest concentration of larvae will be on pasture for potential ingestion by grazing animals.

2.2.4 Seasonal variation in nematode population

Differences in climatic conditions and geograpgical region determines which nematode parasites are prevalent. For example, *H. contortus* predominantes in the warmer climates and *T. circumcincta* is more prevalent in the temperate regions, whereas *T. colubriformis* and some *Nematodirus* species

occur almost everywhere with varying importance (Hervé et al., 2003; Morgan & van Dijk, 2012). Even within one geographical region, both parasite population numbers and species are variable due to complex interactions between various factors (Leathwick et al., 1992) such as the immune status of animals, and farm management decisions involving stocking rates, species of animals kept, and parasite control options. With over 90% of the parasite population suggested to exist outside the host (Familton & McAnulty, 1997) despite expected variations with time, the potential for disease outbreaks is dependent on the timing of availability of peak larvae on pasture, which is syncronised with nematode egg deposition by grazing animals and their successful hatching and development. Populations tend to be overdispersed with a small proportion of animals in a flock usually producing a majority of nematode eggs (Stear et al., 2009) which are unevenly distributed on pasture. This spatial heterogeneity in faecal deposition results in areas around faeces being highly contaminated with infective larvae (Hutchings et al., 2007; Sykes, 1987) with faecal deposition itself highly aggregated throught out a pasture. Variation in weather patterns within season determines the speed and extent of egg development, subsequent hatching and larvae development (Rattray, 2003; Southcott et al., 1976; Vlassoff et al., 2001) resulting in the commonly observed seasonal peaks and troughs in larvae availability. This also reflects the seasonal variation of host susceptibility which in part may be due to events such as parturition or lactation (Roberts & Grenfell, 1991) from immune relaxation during this time.

Lambs are reported to be the main source of nematode egg contamination and provide a substantial contribution to the proportion of larvae on pasture (Leathwick et al., 1998; Vlassoff et al., 2001) as they become readily re-infected. Other sources include nematode eggs from adult ewes (Familton & McAnulty, 1997) and eggs that failed to develop in winter but survived e.g. eggs of *Nematodirus* Spp. (van Dijk & Morgan, 2008; Vlassoff, 1976, 1982). For *Nematodirus* specificaly, Brunsdon (1967) found the infection available to each crop of spring-born lambs resulted from eggs deposited on pasture during the previous spring, summer and autumn.

Knowledge of the GIN parasite epidemiology (Barger, 1999) and factors positively influence their development and survival is key in directing measures aimed at reducing the transmission of infection. This potentially means reducing egg deposition onto pasture by grazing animals through management decisions at farm level. Use of safe pasture is one option which is aimed at breaking the parasite's life-cycle by moving animals to new pasture before parasite larvae develops to the infective stage. Although considerable variations exists between studies on the length of spelling period (Colvin et al., 2012; Colvin et al., 2008; Donald, 1967; Earl & Jones, 1996; Eysker et al., 2005; Leathwick et al., 2001; Rocha et al., 2008), a rest period of between 2-6 months (Bisset et al., 1991a; Rattray, 2003) is thought to be sufficient. Other options include periodic removal of high egg shedding animals (Roberts & Grenfell, 1991).

Alternatively, a sustainable method is to selectively breed for animals with enhanced resistance (Bisset et al., 1996a; Bisset et al., 1994; Miller et al., 1998; Morris et al., 2010; Morris et al., 2000) which may offer epidemiological advantages of reduced pasture contamination.

2.2.5 Ruminant grazing behaviour

Grazing animals are constantly challenged with nematode parasites when grazing on infected pastures. Pasture swards around faeces are often contaminated with greater numbers of infective larvae (Hutchings et al., 2007; Sykes, 1987) and pose an increased threat to grazing animals. The willingness to graze close to the faecal patches or avoidance of such patches can influence the extent of exposure and potential infections (Cooper et al., 2000; Fox et al., 2013; Hutchings et al., 2007; Hutchings et al., 1998; Hutchings et al., 2003; Hutchings et al., 2001; Illius & Gordon, 1990; Seó et al., 2015) particularly when such contact coincides with emergence of L3 (Familton & McAnulty, 1997). Unfortunately, ruminants cannot detect infective stage (L3) larvae on pasture and the presence of faeces has been suggested as a warning signal indicative of the presence of parasites which aids animals to initiate avoidance behaviour (Cooper, 1997; Cooper et al., 2000; Hutchings et al., 1998). Contamination of pasture swards with faeces initiates grazing avoidance due to smell (Aoyama et al., 1994; Dohi et al., 1991) and this odour could be more pronounced in faeces from parasitised animals due to increased leakages of nitrogenous compounds from the damaged digestive tissues (Bown et al., 1991; Poppi et al., 1986). The greater and unattractive olfactory properties of faecal contaminated pasture (Hutchings et al., 2000) and an animal's association of discomfort of previous parasitism with ingestion of faecal-contaminated pasture may enhance the avoidance grazing behaviour. There is evidence of associative learning in animals involving the integration of odour, feed appearance and its taste with associated post-ingstive feedback (Provenza et al., 1992). The unpleasant sensations may, in part, explain aversion behaviour by ruminants over time. In addition, the increased risk of further ingestion of parasites in animals with existing parasitism may help to reinforce the avoidance of pasture contaminated with faeces compared with parasite naïve animals (Hutchings et al., 1998; Hutchings et al., 1999) and may end with less risks. Sheep with an indication of higher levels of infection showed a greater avoidance of faecal swards than animals with lower infection rates (Hutchings et al., 2002), suggesting that animals physiological state may assist with the recognition of the threat of parasite infection. When castrated male Scotish Blackface were trickle-infected daily with 1500 *Ostertagia. circumcincta* larvae (L3) for 15 days, Coop et al (2000) reported that where patches were highly aggregated, infected animals spent greater portion of their total grazing time in uncontaminated patches compared with non-infected counterparts.

Faecal ('parasite') avoidance behaviour would be beneficial to host animals with very limited ability to mount an effective immune response as well as to assist with avoidance of parasites that have

quick developmental time on pasture (Fox et al., 2013). However, animals with greater levels of faecal avoidance run the risks of potential failure to ingest enough nutrients which, if sustained, could potentially result in a nutrtitional disadvantage. Herbivores select non-contaminated swards, when available, over faeces contaminated swards (Cooper, 1997; Forbes & Hodgson, 1985; Hutchings et al., 1998; Marten & Donker, 1964). However, as areas of non-contaminated sward mass declines with time, animals become less selective, resulting in forced grazing of faeces-contaminated swards (Hutchings & Harris, 1997) although parasitised animals still maintain a risk averse strategy (Hutchings et all, 2001) by consistently grazed further away from the soil surface and taking lighter bites at reduced rate.

Faecal avoidance grazing behaviour varies between animals with varying levels of resistance to nematode parasites. Genetically resistant Perendale-line animals were reported to have a greater unwillingness to graze closer to the faecal patches compared with their susceptible counterparts (Hutchings et al., 2007) prompting the authors to suggest that immune function and avoidance may have been co-selected in breeding programmes. Previously, parasite-naïve and immune animals were reported to exhibit lesser faecal avoidance than their parasitised but susceptible counterparts (Hutchings et al., 1998; Hutchings et al., 1999). In contrast, Seó et al. (2015) found that susceptible cattle with higher levels of parasitism grazed further away from faecal contaminated patches compared with resistant counterparts. Immune animals have also been observed to reduce their avoidance of faecal-contaminated patches with time, becoming non-selective (Hutchings et al., 1999; Hutchings et al., 2001). The gradual change of grazing behaviour leading to non-selective grazing could be a result of a learned association between parasitism and the need for increased host nutrition requirements for mentainance and immunity or that resistant animals become aware of their immune status derived from previous infection experiences and, therefore, are increasingly more willing to graze close to faeces. Acquisition of immunity is known to be nutritionally costly to the host animal (Greer et al 2005; Coop and Kyriazakis 2001) and follows a threshold of antigen stimulation in response to the magnitude of parasite challenge. The levels of associated learning between faecal contamination and risks of parasitism in respect to avoidance, would be expected to differ between selection lines and / or individual animals depending on the timing of commensuration of immune recognition. The magnitude of faecal aversion would also be expected to increase in environments where pasture is abundant which could result in heterogeneous sward struture of gaps (in grazed areas) and plumes of grass around faeces (in avoided areas). But with time, there will be a reduction in availability of clean pasture and eventually the only resource present being the swards around or contaminated with faeces, which, by this time may coincide with emergency of infective larvae (Familton & McAnulty, 1997; Fox et al., 2013). Together, these could pose the animal with increased nutrition versus parasitism choice (Fox et al., 2013; Hutchings et al.,

2007; Hutchings et al., 2003) with the trade-off thinly laying between the animal's percieved costs and benefits of grazing around faeces.

2.2.6 Parasitism and animal productivity

Parasitism affects animal productivity primarily through compromising growth in animals responding to infections. Mature worms and the early larval stages can cause major damage to the host (Coop et al., 1982; Sykes, 1997; Symons et al., 1981), the consequences of which include reductions in growth, milk and wool production (Coop & Holmes, 1996; Sykes, 1994), breech soiling and other ailments (Besier & Love, 2003), and mortalities from anaemia induced by *H. contortus* (Van Wyk & Bath, 2002). The magnitude of these effects depends on the rate of infection, parasite species involved (Parkins & Holmes, 1989; Steel, 1978), age, nutrition and resistance status of host animal. Coop et al. (1982) demonstrated the impacts of level of infection on liveweight gain of Blackface x Border Leicester lambs as shown in Figure 2.1. The reduction in liveweight gain was directly proportional to the dose rates, being 10%, 25%, and 47% in lambs infected with 1,000, 3,000 and 5,000 *O circumcincta* larvae per day. Sykes and Coop (1976) had earlier reported that daily infection with 2,500 *T. colubriformis* L3 larvae for 14 weeks reduced weight gain in parasitised lambs by 50% compared with uninfected pair fed counterparts. Symons et al. (1981) reported that weekly infections with 37, 500 and 120,000 *T. colubriformis* L3 larvae significantly reduced mean liveweight gains of 3 - 4.5 months old lambs by 35.3% and 52.9%, respectively, compared with uninfected counterparts by week 12. Similarly, Steel et al. (1980) reported greater depressions in liveweight gain particularly between weeks 8-12 being 67%, 61% and 47% in treatment groups dosed with 3000, 9500 and 30000 infective larvae of *T. colubriformis* relative to unchallenged controls.

A reduction in animals voluntary feed intake is a major factor contributing to the reduced performance often observed in parasitised ruminants (Sykes & Coop, 1976, 1977; Symons et al., 1981) and avoidance of nutritionally rich but faecal contaminated swards does contribute to the reduced intake by grazing animals as highlighted in section 2.2.5 above. Symons et al. (1981) reported that reductions in food intake coincided with the emergence of young adult worms from the abomasal glands. Typically, reductions in feed intake of between 10 and 30% are common (Coop et al., 1982; Dynes et al., 1991; Sykes & Coop, 1976, 1977; Sykes et al., 1988; Symons & Hennessy, 1981) with no overt clinical signs of infection present. However, Steel et al. (1980) reported reductions in food consumption of up to 56% in lambs challenged with 30,000 *T. colubriformis* infective larvae between 8 and 12 weeks of dosing. Severe and complete inappetence has also been reported (Fox et al., 1989). It has also been suggested that animals which are more susceptible to parasitic infection are likely to exhibit more anorexia compared with resistant ones (Sandberg et al., 2006; Zaralis et al., 2008). Reduced voluntary food intake also decreases the efficiency of food

utilization as a consequence of maintenance requiring a greater proportion of ingested nutrients (Coop & Kyriazakis, 2001). Parasitism induces an increase in the loss of endogenous protein into the gut lumen (Bown et al., 1991; Poppi et al., 1986), with the quantitaties varying from about 20 to 125 g metabolisable protein (MP) per day during infection with *T. colubriformis* (Poppi et al., 1986). Overall, infections cause a net diversion of protein away from production into the synthesis of plasma protein as well as in the repair of the gastrointestinal (GI) tract and mucus secretion (Bown et al., 1986; Steel et al., 1982; Symons, 1985; Van Houtert & Sykes, 1996). Estimates suggest that about 4-5 g N per day leaves the terminal ileum (Bown et al., 1991; Kimambo et al., 1988; Poppi et al., 1986), with the inability of animals to replace this increased loss of protein suggested as being responsible for the reduced protein retention often observed in parasitised sheep (Kimambo et al., 1988; Rowe et al., 1988).

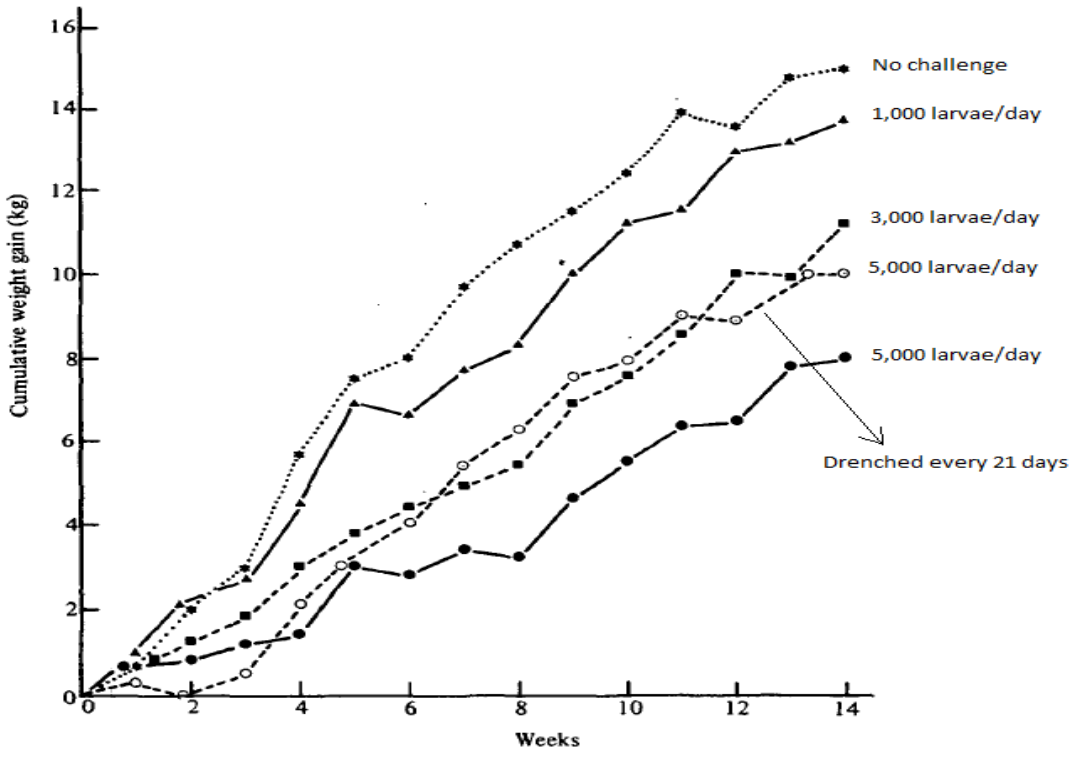


Figure 2:2 Effect of three levels of *O. circumcincta* larvae intake and anthelmintic drench on growth rate of young lambs (modified from Coop et al. 1982)

2.3 Factors that influence development of immunity

2.3.1 Introduction

Immunity provides a state of protection from infectious diseases. The function of the immune system is to provide adequate protection to the host animal against infection while maximising fitness of the individual (Sweeney et al., 2016). To do this, the immune system must recognise the invading organisms and the associated antigens (biochemical protein molecules) they produce. The immune system has both a less specific (innate immunity) and a more specific (adaptive/acquired immunity) component and evolves from the former to the latter. Innate immunity provides the first line of defense against infection. Acquired/adaptive immunity is evoked when an infection eludes or overwhelms the innate defence mechanisms (Janeway Jr. et al., 2005; Lloyd, 1995) but responds to the challenge with a high degree of specificity and memory (Goldsby et al., 2005; Janeway Jr. et al., 2005) which results in a rapid response in subsequent reinfection. Immunity to parasites is acquired as animals are born with no innate immunity to infection. Sykes (2008) postulated that the immune response can be considered to occur in four distinct but interrelated stages: (a) hypo-responsive phase (0-2 months); (b) acquisiton phase 3-4 months); (c) mature phase 5-18 months; and (d) mature-interrupted phase.

A competent immune system is necessary for the healthy life of an animal. A strong active immunity implies that the immunogens (or antigens) recognised by a host, stimulate effective humoral or T-mediated immune responses that do not allow parasite antigen adaptation or evasion (Mitchell, 1979). However, the strength of the immune reaction and the resultant immune response are influenced by various factors including breed, age, gender, prior exposure to the pathogen and physiological status (Watson et al., 1994; Watson & Gill, 1991b).

2.3.2 Antigen stimulus

Exposure to nematode parasite antigens is required for immunity to develop in young lambs and / or in adults for it to be maintained (Douch & Morum, 1993; Jackson et al., 2004). Grazing animals are continuously exposed to various kinds and levels of antigens a consequence of co-infections with multiple parasite species. The immune system must process with speed the perceived threats of infection signaled by the presence of diverse antigens to mobilize mechanisms for appropriate action (Fairlie-Clarke et al., 2009) failure of which would be detrimental to the host animal. Different factors affect the antibodies that can bind to a particular antigen such as the physical conformation and physiochemical properties of the antigen (Janeway Jr. et al., 2005). Further, the level of antigen stimulus also determines the speed of development of acquired immunity to infection in lambs which is dependent on both the magnitude of the parasite burden and the length of exposure (Kambara et al., 1993; Sandberg et al., 2007; Windon & Dineen, 1984).

It is apparent that sufficient antigen stimulus is associated with the initiation of immune development and transition to acquisition phase although there is variability between individuals.

2.3.3 Genetic Factors

Various studies suggest that nematode resistance in sheep is genetically controlled. Some breeds are known to be more resistant to GIN parasites than others (Gray, 1991; Gruner & Cabaret, 1988) and resistant animals tend to respond to infection with greater immunological capacity (Bisset et al., 1996b; Douch et al., 1995; Douch et al., 1994; Green et al., 1999; Shaw et al., 1999) resulting in lesser parasite burdens. The acquisition and expression of immunity is known to be genetically controlled involving many genes (i.e. control is polygenic) (Kemper et al., 2011; Stear & Wakelin, 1998) and varies between breeds and even between individuals within the same breed (McManus et al., 2014; Windon, 1996). Genetic variations are estimated by the heritability coeficient, which is defined as the proportion of phenotypic difference among individuals within a population that is attributable to genetic variation (Falconer & MacKay, 1996). Heritability estimates of host resistance ranges from 0.21 to 0.44 (Morris et al., 1995b; Piper, 1987; Windon, 1996; Woolaston et al., 1990; Woolaston et al., 1991). When examining the degree of variation in resistance to GIN parasites within Merino populations, artificially infected with *H. contortus* or *T. colubriformis*, Woolaston and Eady (1995) reported a greater genetic variation (85%) within-flock compared with 4% and 9% between strains and between bloodlines (pedigree) respectively.

Faecal egg count (FEC) has been used as the most simple and practical live animal indicator trait of relative resistance (Bishop et al., 1996; Bishop et al., 2004; Bisset et al., 1992; McEwan et al., 1992; Morris et al., 2000) and is moderately heritable with h^2 of about 0.34 (Bisset et al 1992), despite FEC being an imperfect measure of parasite burdens (Greer & Sykes, 2012). Analysis of sources of variation among five cohorts of 200 5-months-old Scottish Blackface lambs (Stear et al., 1996) showed that the variation in FEC output could be assigned to eight sources: additive genetic variation (30%), maternal effects (14%), factors unique to each individual (22%), variation in measurement (22%), type of birth [single or twin (5%)], early FEC (4%), sex (2%) and date of birth (1%). Eady et al. (1996) reported that the major source of genetic variation for FEC among merino sheep was within bloodlines which accounted for 22.2% of total variation, with progeny of individual sires showing a wide range in parasite resistance. Further, of the tools available to reduce contamination, Eady et al. (2003) reported the greatest reduction in FEC was acheived by genetic selection (69%) compared with 35% and 28% resulting from protein supplementation and drenching, respectively.

Generally, breeds of sheep and goats that have evolved under continued strong challenges are more resistant to nematode parasites when compared with exotic or less well adapted breeds run in the same environment (Baker, 1995; Baker & Gray, 2004; Bishop & Morris, 2007; Courtney et al., 1985;

Matika et al., 2003; Morris et al., 1995b; Mugambi et al., 1997; Preston & Allonby, 1979). The superior resistance of such breeds, is suggested to have been influenced by natural selection (Stear & Wakelin, 1998) with little or no anthelmintic treatment. For example, the Red Maasai sheep of Kenya are more resistant than imported breeds (Baker et al., 1993; Preston & Allonby, 1978), Florida Native (Windon, 1990; Windon et al., 1993; Woolaston et al., 1995b) are more resistant than other breeds in USA (Bahirathan et al., 1996; Gamble & Zajac, 1992; Miller et al., 1998), St Croix is more resistant than Suffolk, Finn-Dorset, and Rambouillet (Courtney et al., 1985). In many of these examples animals are faced with challenges of the highly pathogenic *H. contortus* in the absence of anthelmintic, as such, immunity is crucial to survival.

Modern techniques in genetic resistance

Molecular techniques such as quantitative trait loci (QTL), genome-wide association studies and candidate gene analysis (McRae et al., 2014; Pemberton et al., 2011) have increased understanding of the genetic mechanisms underlying resistance. Further, identification of regions thought to have undergone "selective sweeps", i.e. beneficial mutation due to selection, can help reveal genes that underly phenotypic differences. In this regard, McRae et al. (2014) identified 16 regions, among which were candidate genes that are involved in chitinase activity and those for the cytokine responses. Crawford et al. (2006) identified significant QTL for parasite resistance at the telomeric end of chromosome 8 which was associated with adult worm burdens, while a QTL for immune functions for serum IgG was identified on chromosome 23. Interferon gamma (IFN- y) gene on the q arm of chromosome 3 has been associated with parasite resistance in Romney selection lines (Paterson et al., 2001) and wild populations of Soay sheep (Coltman et al., 2001). Paterson et al. (2001) reported significant differences in marker allele frequences between resistant and susceptible Romney animals. The frequency of the (IFN)-y ins/del region in the A allele was 0.82 for parasite resistant and and 0.29 for susceptible animals. The authors suggested that interferon gamma marker alleles were segregating with resistance and susceptibility to parasites. Contrary, no evidence of such allelic association was found in Perendale selection-line animals in the same study which could be suggestive that alleles are fixed in Romney lines. While the functional polymorphism in a coding region of the (IFN)-y gene is not yet clear, interferon gamma is a cyotkine associated with important roles in the regulation of immune response to parasite infection (Wakelin, 1996). Diez-Tascon et al. (2005) reported that genes were differentially expressed in resistant Perendale lambs, particularly those known to be involved in acquired immunity as well as those associated with smooth muscles structures in the intestine while in susceptible animals, gene expression appeared to be of a hypersensitive response to stress and stimulus. Coltman et al. (2001) found that FEC was highly associated with alleles at the (IFN)-y microsatellite locus which was suggestive that a QTL associated with reduced FEC may be segregating near the (IFN)-y gene in Soay sheep. The authors concluded

that a functional polymorphism which leads to the reduced expression or efficacy of (IFN)-γ could enhance the immune response to GIN parasites which favours Th2-cell activity and its associated immune mechanisms. In summary, there is abundance of emerging evidence that suggests a genetic basis of resistance to parasite infection and their subsequent roles in differential expression of cytokines that regulate immune responses. Variations in experimental designs and markers used, make direct comparison between studies more difficult.

2.3.4 Age of the host animal

Development of host immunity to GIN parasites appears to be influenced by age of the animal. Growing lambs between 3 and 6 months-of-age acquire immunity to GIN infections more slowly when compared with more than 8-months old sheep (Dineen et al., 1978; Gregg & Dineen, 1978; Manton et al., 1962b; Urquhart et al., 1966a). Consequently, they do not mount a vigorous immune response (Dobson et al., 1990b) and are less able to prevent parasite larval establishment and / or to remove existing burdens (Brunsdon, 1962; Dineen et al., 1978; Gibson & Parfitt, 1972; Manton et al., 1962b; Smith et al., 1985). The situation is further exacerbated by the increased larvae challenge on pasture from the presence of ewes during peri-paturient relaxation of immunity (Williams, 2011). The lesser resistance in lambs, has been attributed to immunological hyporesponsiviness (Colditz et al., 1996; Watson & Gill, 1991b) as young lambs had significantly lower proportion of $CD4^+$ and $CD8^+$ lymphocytes, in blood, lymph and skin compared with mature sheep (3-6 years old). Parasite antigen-primed CD4+ T lymphocytes are known to be associated with protective immunity in older animals (Kambara and McFarlane, 1993) and their depletion resulted in abrogated expression of resistance in genetically resistant Merino (Gill et al., 1993) and Gulf Coast Native (Peña et al., 2006) lambs infected with *H. contortus.* This was suggested to be the result of alteration of the functioning of surface coating of the lymphocytes by antibodies. The deficiency in the immunological machinery was reflected in the magnitue of both humoral and cell-mediated responses. The authors reported that sheep less than 12 moths old have lower antibody responses to a range of antigens (Watson and Gill, 1991b; Watson et al., 1994). However, increasing the antigen dose 3-fold failed to overcome the deficiency in relative immune responses of young sheep despite stimulating greater concentration of antibody (Watson et al., 1994). Similarly, McClure et al. (1998) found no evidence of immunological capacity in young Merino lambs to recognise *H. contortus* antigens following trickle infection. Further, trickle infection did not significantly protect young lambs against *H. contortus* challenge but lambs were only partially protected (45%) by 3-months of age against *T. colubriformis* infection. It is apparent that young lambs lack the immunological ability to respond to antigenic stimulus which is pre-requsite for acquisition of immunity (Gill, 1994; Gill et al., 1993; Greer & Hamie, 2016; Watson et al., 1994; Watson & Gill, 1991b).

Previous exposure to parasite infection has also been suggested to influence the extent of development of acquired immunity. Barger (1988) showed moderate experimental burdens of adult *H. contortus* in young lambs stimulated protective immune developemnt, whereas their suppressively drenched counterparts remained susceptible to incoming infection. Kimambo et al. (1988) reported that re-challenge infection caused a rapid development of eosinophilia within 6 weeks. The apparent absence of nematode eggs during 10 weeks of re-infection suggested that the infective larvae failed to develop into mature worms, either because their development into adult worms was inhibited by the immune response of their hosts or because they were excluded before they could establish, or both (Kimambo et al., 1988). In contrast, Manton et al. (1962b) found no difference in the worm burdens of young Suffolk X Finn Dorset lambs (2-4 months) which had previously experienced infections as previous experience of *H. contortus*, given infections either as two doses of 1,500 larvae or as a trickle infection, failed to protect 2-4-month-old lambs against re-infection with 5,000 larvae. The authors suggested that failure of the lambs to become resistant may have been due to an incomplete development of some part of the self-cure mechanism. Other studies have also reported failure of lambs to acquire resistance to prolonged primary infection with *T. colubriformis* (Chiejina & Sewell, 1974; Gibson & Parfitt, 1922).

What is less clear is whether it is age *per se* or associated factors such as weaning, live weight, fat reserves or nutrition (McClure et al., 2000) that accounts for the inability to develop immunity. There is information to suggest that lambs have some degree of immunocompetence at birth which helps them to respond to a variety of antigens in the neonatal period (Colditz et al., 1996; Watson & Gill, 1991b) although the rate and magnitude are reduced compared with adults. In an experiment to evaluate the induction of protective immunity to infection with *T. colubriformis* in neonatal merino lambs, Emery et al. (1999) reported that young lambs had the capacity to develop protective immunity by 10 weeks-of-age. The authors suggested that suckling provided lambs with optimal nutrition needed for the development of protective immunity against parasites. Valera-Diaz (1970) also reported that lambs infected with *H. contortus* require 7 weeks of infection to exhibit evidence of 'self-cure', as a consequence of increased nutrition. Contrary, Sykes (2008) pointed out that nutrition had little immediate effect on development of resistance in young ruminants or mitigation of costs of parasitism. This lack of effect of nutrition may probably reflected a hypo-responsive immune status, a suggestion which is supported by the lack of the effect of suckling on the ability of lambs to resist *T. circumcincta* infections (Iposu et al., 2008). Weaning has also been shown to temporarily retard the development of immunological response to internal parasites. Gill (1991) reported that suckling lambs developed immunity earlier and had higher serum antibodies to *H. controtus* antigens than weaned lambs and, by the age of 12 weeks, weaned lambs had greater FEC and correspondingly lower packed cell volume compared with unweaned group.

Similarly, Watson and Gill (1991a) found that high (5,000-10,000) burdens of *H. contortus* in lambs weaned at 8-weeks-of-age had twice the mean FEC and had a greater reduction in packed cell volume (25.4 from 35.5) than controls (30.0 from 36.0). The physiological responses associated with the stress of weaning, and particularly the elevation in glucocorticoids, has been found to result in increased susceptibility to pathogen infections (Parrillo & Fauci, 1979). Watson and Gill (1991a) concluded that up to 3-months old suckled lambs have better prospects of mounting a strong immune response than weaned lambs when both are faced with subclinical parasite challenge. Yet other studies have attributed the associated lamb body weight at the time of weaning and / or first encounter with parasite infection as being critical to the development of resistance and or immunity to parasite infection. McClure and Emery (2007) and Dobson et al. (1990c) found that Merino lambs with less than 23 kg of weight at first exposure to *T. colubriformis* showed an impaired ability to develop protective mucosal immunity to rechallenge infection and the failure was positively correlated with plasma antibody and mucosal IgA antibody concentrations.

2.3.5 Nutritional factors

Nutrition is considered to be an important factor in the host-parasite relationship and can influence the impact of GIN infections. Nematode parasites can hydrolyse components of their host tissues (Liu et al., 2003) which can reduce the efficiency of nutrient absorption and utilisation (Sykes & Coop, 1976, 1977). However, it is not clear as to how much of the induced protein deficiency is due to direct parasitic effects or a result of the increased synthesis of protein required for the production of antibodies and to replace endogenous mucus and plasma secretions (Williams, 2011). Overall, it can be expected that in parasitised animals increased protein may be diverted from production fuctions related to growth into synthesis of plasma protein and repair of the GI tract (Bown et al., 1986; Steel et al., 1982; Symons, 1985). This is consistent with the priority nutrient partitioning framework proposed by Coop and Kyriazakis (1999). Although the magnitude of these responses has not been fully assessed, there are suggestions that parasitised sheep may need to synthesise approximately 50 g more protein per day (Coop & Kyriazakis, 1999) suggestive that supplementing diets with high protein feeds could be beneficial.

Nutrition can contribute to regulating parasite populations in a host by either acting independently or through enhancing development of immunity (Coop & Kyriazakis, 2001; Houdijk et al., 2003; Sykes & Kyriazakis, 2007). In a review, Athanasiadou et al. (2009) stated that host nutrition can affect both the resident parasites and / or incoming larval populations and help in reducing the detrimental consequences of an infections in small ruminants. The authors suggested that nutrition can 1) affect the parasite fitness through ingestion of plant secondary compounds, 2) altering the conditions in the gut environment to toxic levels for parasite survival, 3) may positively contribute to the hosts ability

to regulate GIN infections, and 4) enhance host resilience to the parasitic infection. However, the main influence of nutrient supplementation may be more associated with degree of expression of immunity (Balic et al., 2000) as it is likely given a lower priority for a scare resource allocation than the functions of maintenance, growth or reproduction. With adequate nutrition a host can more readily cope with the consequences of parasitism and may eventually overcome their effects (Coop & Kyriazakis, 2001). Generally, the improvements in resilience resulting from dietary protein supplementation are greater in young naïve animals in which the pathophysiological disturbances to the GI tract are often most pronounced (Fox, 1997; Holmes, 1993). Further, sheep continously dosed with *T. colubriformis* were found to alter their diet and systematically increased selection of a greater proportion of a high protein food than uninfected controls (Kyriazakis et al., 1994) indicating the ability of animals to recognise the increased protein requirements imposed by infections.

Some studies have, however, suggested that the provision of additional dietary protein does not affect the initial establishment of nematodes and / or early acquisition of resistance in young sheep (Abbott et al., 1985; Bown et al., 1991; Coop et al., 1995; Van Houtert et al., 1995). In a study to determine whether suckling offers protection to lambs against *T. circumcincta* infection in lambs upto 12 weeks of age, Iposu et al. (2008) found that continued suckling appeared to have a major benefit of providing nutrient for enhanced growth and / or resilience to infection although lambs may have been too young to suffer the effects.

The presence of some compounds in plants, particularly secondary metabolites consumed by herbivores (Athanasiadou et al., 2003), also have influence in reducing the negative impacts of parasitism although these effects may be a consequence of direct anthelmintic effects rather than solely enhancing resilience through increased protein supply. It has been reported that condensed tannins (CT) control GIN by interfering with hatching of parasite eggs and their development to infective stage larvae (Min & Hart, 2003). Evidence for direct anthelmintic effects of CTs has been shown by drenching sheep infected with *H. contortus*, *T. circumcincta* and *T. vitrinus* which reduced the viability of infective larvae for all three species (Athanasiadou et al., 2001). In addition, there was a strong linear relationship between larval viability and concentration of CT from Quebracho extracts; 1.00, 0.992 and 0.974 for *T. circumcincta, H. contortus* and *T.vitrinus*, respectively. Similarly, Molan et al. (2000) demonstrated that CT extracted from big trefoil (*L. pedunculatus cav,*), birdsfoot trefoil (*L. corniculatus L.*), *H. coronarium*, and sainfoin (*O. viciifolia* Scop.) forages had dose-dependent anthelmintic effects against immature stages of several nematodes. Similarly, feeding Serecea Lespedeza *(Lespedeza cuneata, SL)* has been reported to have inhibitory effects on larval development in the faeces and adult worms in goats infected with *H.contotrus, T. circumcincta and T. colubriformis* (Shaik et al., 2004; Shaik et al., 2006).

Hay from SL was effective in reducing FEC by 67-98% and worm burdens by 62.7% in goats infected naturally and / or experimentally with *H. contortus* (Lange et al., 2006). In a confined study, Terrill et al. (2009) found that goats fed 25%, 50% and 75% of SL hay supplement, had 45.5%, 66.3% and 74% lower FEC than the control group which was fed 75% bahiagrass.

2.3.6 Relative/physiological Maturity

Given that age and weight are confounded, it has been suggested that capacity to develop immunity may be associated with relative maturity. Similarity in the growth pattern of different species indicated that the age at which a species is expected to attain a given degree of maturity in body weight was proportional to the 0.27^{th} power of its expected mature weight (Taylor, 1965, 1968). The implication of the standardised growth curves is that the expected degree of maturity at a given metabolic age and the expected metabolic age at a given degree of maturity can be extrapolated and compared. Metabolic age is defined as the age in days of an animal from an origin near conception and when expected mature body weight is scaled to the power 0.27. In contrast, the degree of maturity is the proportion of the animals immature weight at any point in time to its expected mature weight. Some species are known to be earlier maturing in body weight than others (Taylor, 1980) although variations exist in time taken to mature even among animals of similar mature weights. (Taylor & Fitzhugh, 1971) reported genetic correlation between time taken to mature and mature weight of 0.34, 0.41, 0.39 and 0.39 at birth, 6, 12 and 18 months of age, and 0.48 for mean time taken to mature. However, animals that are early maturing can be expected to have a lighter body weight than animals that take longer to mature. Taylor (1985) stated that genetic comparisons made at the same metabolic age or at the same degree of maturity are independent of adult size and such comparisons eliminate all-pervasive influence of body size when comparing species or genotypes in terms of immature physiological characteristics.

Animal growth can be expected to result in substantial changes in body composition and the proportion of lean and body fat (Delavaud et al., 2007; Delavaud et al., 2000; Greer & Hamie, 2016; Greer et al., 2009a; Pittroff et al., 2008) particularly between weaning and pre-pubescence when growth accelerates. Considerable variations in the quantity of fat deposition and distribution in the fat depots can be influenced by breed, sex and / or diet (Blache et al., 2000; Delavaud et al., 2007; Delavaud et al., 2000; McClelland et al., 1976). The increase in mass and proportion of adipose tissue in the carcass, can be expected to result in a corresponding increases in circulating adipocytokines (Ahima et al., 1997; Blache et al., 2000; Boisclair et al., 2000; Greer et al., 2009a; Lord et al., 1998). Among the many adipocytokines, leptin has been shown to have a modulatory role in T-cell immune responses in mice (Lord et al., 1998) as well as in signalling the current nutritional state of the animal (Ahima et al., 1997; Ahima et al., 1996).

Thus, an animals physiological state may help in understanding the timing of immune development as an increase in physiological/relative maturity may be expectecd to help in heightening the adipocyte-derived signals for the initiation of immune development during the acquisition phase.

Implications of relative maturity

Several implications can be drawn from the phenomenon of physiological maturity but three are worth mentioning here in the context of this review. First, animals with a lighter mature body weight will be expected to fit all the life events within their shorter lifespan as time variables scale to the power of 0.27. This necessitates an increased rate of tissue development which would result in animals that are physiologically more mature at the same chronological age compared with animals with heavier body weights (Greer & Hamie, 2016). These lighter, but physiologically more mature animals would be expected to exhibit greater immunological capacities early in their lifetime (Douch et al., 1996) as development of immunity increases with age (Coop et al., 1982). Secondly and by extrapolation, the often observed susceptibility of male animals to nematode infection relative to their female counterparts at same chronological age (Barger, 1993) may suggest the physiological differences in relative maturity owing to the higher mature weights in males and the longer chronological time required to achieve this. Third but not least, immune development is associated with nutritional costs, particularly protein (Greer, 2008), thus early immune development may help explain the often observed reduced live weight in Romney and Perendale lines selected for resistance (Bisset et al., 2001; Morris et al., 2000; Morris et al., 1997; Morris et al., 2005; Watson et al., 1992) as well as in breeds considered to be naturally resistant from environments where the highly pathogenic *H. contortus* is dominant (Riley & Van Wyk, 2009; Van Wyk & Bath, 2002) as these animals may be physiologically more mature at the same chronological age.

2.3.7 Costs of development of immunity

Improving immunity can provide benefits to the individual, in terms of expulsion of nematodes and reducing the susceptibility of the flock to reinfection through reduction in pasture contamination (Bisset et al., 1997a; Greer, 2008; Laurenson et al., 2012; Miller et al., 1998). However, the direct benefit of immunity to the performance of the animal may not be apparent (Greer et al., 2008; Greer et al., 2005) until sufficient time is allowed for animal to recoup the nutritional investments of immune development (Greer, 2008). In addition, it has been suggested that a trade-off exists in resources deployed between the cost of diverting host defence mechanisms towards an active immune response and the risk of eliciting too strong an immune activity (Graham et al., 2005). Colditz (2008) identified six potential costs to the host from immune activation and these are (i) increased metabolic activity; (ii) reduced nutrient availability due to anorexia; (iii) altered priority for nutrient utilization; (iv) change in size and turnover of pools of immune cells and proteins;

(v) immunopathology from inappropriate or excessive immune activation; and (vi) genetic cost that arises from a change in the capacity of offspring to express production and life-history traits following selection for parasite resistance.

The acquisition and maintenance of immunity to gastrointestinal parasites in sheep is nutritionally costly (Greer, 2008; Greer et al., 2005). Sheep incur an estimated 15% loss of productivity (Sykes, 1994) and 16% reduction in effeciency of nutrient utilisation (Greer et al., 2005). Klasing and Calvert (1999) reported that 60% of the decreased growth during an immune response could be attributed to activities of the immune system while 40% was due to indirect effects, such as inappetence. Similarly, Dever et al. (2016) reported 75% of the overall costs of infection with *T. colubriformis* L3 larvae were derived from the hosts immunological responses during the first 35 days of infection. Moreover, Blackburn et al. (2015) also reported that immune response accounted for 39% of the reduced growth rate in cross-bred Border Leicester/Merino X Poll Dorset meat lambs infected with *T. vintrinus*. Overall, these studies suggest that the immunological machinery requires substantial nutritional resources which an animal must invest to assure survival, which must be recouped to allow for greater production.

2.4 Selection for resistance and/ or resilience

2.4.1 Selection for Resistance

Selection (natural or artificial) is a process of influencing the relative contribution of reproductive success (fitness) of a genotype(s) and its associated phenotype(s) to the next generation.

A selection programme for resistance (low FEC) and susceptibility (high FEC) in New Zealand involving Romney sheep, was established by Agresearch in 1979, and has been maintained since (Bisset et al., 1996a; Morris et al., 2010; Morris et al., 1995b). Romney sheep were actively selected within lines based on faecal egg count (FEC) which is moderately heritable (Bisset et al., 1992; Morris et al., 1995a; Morris et al., 2000; Morris et al., 1997) and other measures of resistance were incorporated later such as immunological responses and modern marker assisted selection. The objective was to provide alternative and more sustainable nematode parasite control options to anthelmintics based on the premise that resistance evolves faster if dominant, slower when co-dominant and least when it is recessive (Barnes & Dobson, 1990; Dobson et al., 1996). Initial stages involved small scale studies of 30 low-FEC (resistant) and 30 high-FEC (susceptible) animals screened from approximately 300 field-grazed ewe lambs (Baker et al., 1990; Bisset et al., 1991b) but later, both ewe and ram replacements for the lines were selected from in-bred progeny (Bisset et al., 1991b). In 2008, the lines were transferred to Lincoln university from which point they were selected within line without anthelmintic treatment and replacement ewe and ram lambs were randomly selected within each line each year (Greer et al., 2018). Most of these selection studies used parasite infections acquired naturally from pastures (Bisset & Morris, 1996; Bisset et al., 2001; Bisset et al., 1996a; Morris et al., 2010; Morris et al., 2000; Morris et al., 1997; Morris et al., 2005). In contrast, most resistance selection studies in Australia have used artificial challenge with nematode species (Albers et al., 1987; Woolaston & Baker, 1996; Woolaston et al., 1995a; Woolaston & Piper, 1996; Woolaston et al., 1991) whereas in other regions a mixture of both natural and artificial infections have been utilized (Amarante et al., 1999a; Baker et al., 2003b; Good et al., 2006; Manton et al., 1962a; Matika et al., 2003; Mugambi et al., 1997; Rowe et al., 1988; Van Wyk & Bath, 2002; Vatta et al., 2002). Faecal egg count (FEC) has been the mostly widely used indicator trait for determining resistance principally because of its convenience although imperfect. Other measures include packed cell volume, eosinophils, immunoglobulins, CarLA, FAMACHA, worm burdens etc. One notable observation is that resistance to nematode parasites is not binary, rather it evolves with time and influenced by various factors such as antigen stimulus, host age, genetics, nutrition and physiological maturity, as detailed in Section 2.3. With these measures, however, resistant animals have been identified, relative resistance assessed and compared between and within breeds, and in some cases used in cross-breeding programmes to improve responses of animals perceived to be less resistant / adapted to handle the effects of parasites (Amarante et al., 1999b; Amarante et al., 2004; Baker & Gray, 2004;

Gamble & Zajac, 1992; Good et al., 2006; Gruner et al., 2003; Matika et al., 2003; Mugambi et al., 1997). The process of effecting resistance is expensive and time consuming (McEwan et al., 1997) as the trait is controlled by many genes i.e. polygenic. It is on this basis that modern techniques have gained ground in trying to speed up the process of understanding the mechanisms underlying resistance, a synopsis of which is provided in Section 2.3.3.

Sheep improvement limited (SIL), is a part of Beef + Lambs New Zealand Genetics and it provides tools and information for ram breeders and commercial farmers aimed at improving the profitability of New Zealand sheep farming. Using selection indexes, both resistance and production can be improved simultaneously. Selection index combines breeding values from different traits to determine best overall genetic progress. Breeders then use these selection indexes to aid in understanding the overall genetic merit of an animal across key traits using a common scale, usually in dollars or cents (Beef + Lamb NZ, 1999; Young, 2012). In sheep for example, traits such as weight of lambs at weaning, wool production, carcase yield percent (a measure of post-weaning growth), faecal egg count (measure of resistance) and other health traits, reproduction (litter size) and lamb survival etc, are included in the selection indexes. For every analysis, SIL uses 1995 as the benchmark year and average genetic merit of lambs are compared with 1995 average to allow progress to be assessed

Selection indexes for both resistance and production traits was started in 1994 and is available to commercial ram breeders through the WormFEC™ Service (McEwan, 1994; McEwan et al 1995). WormFEC provides advice on the challenge protocol and allows for breeding values to be calculated including sire referencing where appropriate. Both FEC and antibody levels are log transformed prior to analysis and FEC indices back-transform and results expressed as percentage of the average FEC of the flock. Under the WormFEC protocol, sample lambs are tested for *Strongyle* (FEC) and *Nematodirus* (Nemat) at weaning (FEC1, Nemat 1) and at about 8 months (FEC2, Nemat2) and when sample FEC levels approaches 800 epg and 1500 epg on commercial farms and research flocks, respectively (Beef + Lamb NZ, 1999), then each lamb is tested for FEC and live weight. Sires identified from these tests, with combined highly productive progeny and marked resistance to internal parasites challenge are supplied to breeders for matting with most productive and parasite resistant females (McEwan et al 1995).

The selection indexes are not without shortfalls. It can be expected for example that two animals may have similar indexes for overall merit. One animal may exhibit good performance across all traits while the other may have exceptional merits only in one or two traits. This can mask the average to poor performance in other traits (Young, 2012) giving such animals a change to be selected.

The other shortfall is that indexes can be dominated by a few traits with high economic merit, but these may not be what the Breeders want to focus on, although SIL has optimised selection indexes for most sheep farms for dual-purpose and terminal sires.

2.4.2 Selection for resilience

An alternative to selecting for increased host resistance is to select for animals which are resilient i.e. animals that maintain high production even under low to moderate prolonged challenge but requiring less drenching relative to susceptible control groups. In New Zealand, genetic selection for resilience commenced in 1991 but initially involved progeny rams (n=213) selected from 5 different commercial Romney ram breeding flocks (n=14,000) within AgResearch experimental flocks (Bisset et al., 2001). Due to the challenges associated with measures of resilience (Bisset & Morris, 1996), the use of a drench-on-demand procedure was designed to drench only animals deemed to require anthelmintics based on body weight and condition score and/ or breech soiling (dags). With this approach, lambs that were more resilient could be challenged for prolonged periods, with moderate to high infections with no anthelmintic treatment but avoided risking the health of the least resilient ones (Bisset et al., 1996a; Bisset et al., 1994) and the approach has widely been adapted and / or modified to suit different circumstances in various geographical regions (Bath & Van Wyk, 2009; Bath, 2014; Besier et al., 2010; Busin et al., 2014; Greer et al., 2009b; Stafford et al., 2009; van Wyk et al., 2006). Overall, selection for resilience trait is possible, although slow, (Bisset & Morris, 1996; Bisset et al., 1996a; Bisset et al., 1994) but under New Zealand conditions, the trait appears to be independent from resistance (Bisset et al., 2001; Bisset et al., 1996a). However, selection for resilience if based on individual performance or drench requirements may not be practical because of low heritability (0.10-0.19) (Bisset et al., 2001; Bisset et al., 1996a). Resilience is confounded by the need for prolonged exposure to parasites to assess animals' ability to withstand worm challenge versus the expected genetic potential for growth *per se*. Further, selection for resilience in dual-purpose breeds in New Zealand may not reduce FECs (Bisset et al., 2001; McEwan et al., 1997). The main benefits of resilience animals may be expected through their improved ability to maintain health and acceptable productivity under challenge. It is suggested that benefits of resilience can be optimized through use of a selection index in identifying animals that show both resistant (low FECs) and high productivity in the face of parasite challenge as described under Section 2.4.1 above.

2.4.3 Prediction models

The relationships between parasite populations dynamics on pasture influenced by selection progress has been explored *in silico*. In a review of models of gastrointestinal parasite populations, Smith and Grenfell (1994) highlight some of the shortfalls and challenges associated with the approaches.

One of the key challenges suggested is the difficulty of model validation; that it is difficult to compare with field data. The other difficulty highlighted is that as more biological details are included, the modelling for several crucial elements becomes progressively difficult. As such, it has been suggested to adopt a rational stepwise fashion of adding components and evaluating model behaviour at each stage until it becomes adequate (Smith & Grenfell, 1994). A few of the developed models are presented here for purposes of elaboration. Dobson et al. (1990a) developed a model to predict worm population over time as a function of infection rate and host age considering that host acquired immune responses are important in regulating parasite populations. It rested on the assumption that a threshold of nematode burdens must be reached before resistance begins to develop and it predicted a declining rate of establishment of adult *T. colubriformis* worms with time once host immune responses were sufficiently stimulated. Leathwick et al. (1992) predicted that the pattern of parasite dynamics within the host can largely be explained by the ability of the host to resist infection with immunity resulting in lower pasture larvae contamination. Using data from two independent experiments, the model by Leathwick et al. (1998) estimated the production benefits of breeding for resistance to nematode parasites, with estimates of 3.0 kg gain in live weight and 0.3 kg increase in fleece weight at 12 months of age expected from reducing FEC by 50%. When host resistance was included in the model together with production traits, the performance estimates increased by more than 20%. Bishop and Stear (2003) modelled host genetics and resistance to diseases caused by nematode infections and predicted that selection for resistance in sheep should be successful in reducing pasture contamination and subsequent larvae challenge due to the expected reduction in worm egg output. The authors suggested that genetic relationships between resistance and performance are difficult to predict despite the impacts of infection on performance. Laurenson et al. (2012) concluded the epidemiological consequences of resistance to GIN parasites would be substantial from grazing resistant animals separately as opposed to grazing sheep of different resistance to GIN parasites together. The model predicted 8.5-fold higher FEC in susceptible group compared with the resistant lambs. Using the model to simulate the effects of grazing lambs of different resistance status grazing separately or together with the procedure repeated for 3-grazing seasons, the authors reported a predicted reduction in average pasture contamination of resistant group by approximately (~) 83% whereas the susceptible group increased contamination by ~240% compared with the whole population average. Further, Fox et al. (2013) have recently modelled parasite transmission in grazing systems by integrating animal grazing behaviour, immunological responses and other transmission processes, such as development of larvae within host and on pasture, spatial heterogeneity of pathogens and nutrition resources among others. The authors showed that susceptible animals accounted for the rapid increase of ingested larvae as well as adult parasites in the host animal, but parasite burden was subsequently predicted to decline due to the acquisition of immunity.

The model further predicted that grazing behaviour would greatly affect parasite transmission by influencing both the timing of animal's exposure to larvae and magnitude of such an encounter. Together, these models suggest that dynamics of larval population is highly variable but transmission and potential disease outbreaks are associated with peak larval contamination on pasture which can be reduced by incorporating resistance traits in grazing animals as a control strategy that would minimise drug usage and slow development of anthelmintic resistance by parasites.

2.4.4 Benefits of selection for resistance

Epidemiology predictions

Selection for resistance has been successful in reducing nematode FEC (Bisset et al., 1997a; Laurenson et al., 2012; Miller et al., 1998; Morris et al., 1995a; Morris et al., 2000) which could potentially reduce pasture contamination with infective larvae and make healthier grazing environments for subsequent grazing. In lines of Romney sheep, selection for resistance is reported to have considerably reduced both FEC and worm burdens (Bisset et al., 2001; Bisset et al., 1996b; Bisset et al., 1991b) resulting in progeny from the most resistant Romney rams harbouring 70% and 85% fewer worm burdens and FEC, respectively, of a majority of economically important sheep nematodes species compared with susceptible counterparts (Bisset et al., 1991b; Morris et al., 1995b). Morris et al (2000) also reported that resistance reduced FEC by 11-fold difference compared with the high-FEC (susceptible) selection line involving the 1993-1994 crop of lambs. In environments where the highly pathogenic nematode species such as *H. contortus* are predominant, resistant animals have been associated with lower FEC, higher packed cell volume or low FAMACHA scores (Amarante et al., 1999b; Amarante et al., 2004; Gamble & Zajac, 1992; Matika et al., 2003; Mugambi et al., 1997; Van Wyk & Bath, 2002; Vatta et al., 2002). Such large reductions in FEC would lead to slow larvae build-up on pasture. The few initial studies where lines have been run separately appear to be consistent with the greater epidemiological benefits anticipated with selection for nematode resistance. In the US for example, the Gulf Coast Native sheep and their cross-bred F1 generations (Li et al., 2001; Miller et al., 1998) were reported to have consistently maintained lower FEC when grazed separately from the susceptible Suffolk breed in three consecutive years. In addition, the resistant Gulf Native lambs were found to have fewer *H. contortus* eggs compared with Suffolk lambs suggesting a greater capacity of the native lambs to control infection of the more pathogenic nematode. In New Zealand, few farmlet based studies have been undertaken which have reported substantial pasture larval contamination in farmlets separately grazed by susceptible animals compared with those grazed by resistant Romney lambs. Bisset et al. (1997a) reported that farmlets grazed by susceptible lambs had approximately (~) 5-6 fold greater nematode larvae infestation on pasture compared with those grazed by resistant counterparts suggesting that the degree of resistance to *Trichostrongyle* infection in lambs has a substantial impact on the build-up of larval

infestation on pasture. Leathwick et al. (1998) reported that grazing genetically resistant and susceptible Perendale flocks on separate farmlets resulted in more than three-fold differences in levels of pasture larvae and 9 to 13-fold differences in lamb and ewe FECs. Similarly, Morris et al. (1997) reported 6.0 and 6.4 fold differences in pasture contamination between susceptible and resistant female lambs of 1990 and 1991 when grazed separately.

In most commercial breeding and / or farming situations, resistant and resilient or susceptible line animals may not be run separately. So although selection for resistance appears to have the potential to reduce larvae contamination on pasture through greater removal of L3 by ingestion than excretion of eggs in faeces than susceptible animals, the overall pasture contamination may not be expected to change considerably simply by the presence of a small number of resistant animals in a flock (Bisset et al., 2001; Sutherland & Scott, 2010). Conversely, grazing selection lines with differing responses to parasites, will mask the expected impacts of selection for increased resistance. In contrast, solely grazing animals selected for resistance would reduce pasture contamination which may lead to improved productivity.

Production benefits

Predicted benefits of selection for nematode resistance on production have not always been realized. Correlations between FEC and liveweight gain or breech soiling in lambs under nematode challenge has not been consistent. Morris et al. (1997) reported that weaning weights were not different between resistant and susceptible lambs and that resistance had no apparent advantage over susceptibility in either post-weaning liveweight gain or autumn weights while the lines grazed together. A continued selection of Romney sheep for resistance and susceptibility to nematode infection by (Morris et al., 2000), summarised in Table 2.2, reported no difference in weaning weights, post-weaning gain and Autumn weights for lambs born between 1985-1997 with exception of the period between 1993-1997 which suggested a difference in favour of susceptible lambs. Worth mentioning is that in all these years (1985-1997) resistant and susceptible lines animals were grazed together. This also lead Morris et al. (1995b) to conclude that genetically resistant lambs have no apparent growth rate advantage over the high FEC counterparts when grazed together. Similarly, Morris et al. (2005) reported that genetically resistant Perendale line animals were 6-12% and 24-26% lower in body weight and fleece weight, respectively, compared with their high-FEC line counterparts.

Initial studies of selection for resilience selection reported that the trait is achievable despite a low heritabilities of between 0.21-0.35 (Bisset et al., 1996a; Bisset et al., 1994). The authors suggested that although genetic correlations were low, the approach would lead to lambs with better growth rates under challenge. Other studies have reported that selection for resilience resulted in an

increased post-weaning weight gain of up to 27% while concurrently reducing the number of anthelmintic treatments by 25-50% (Greer et al., 2009b; Morris et al., 2001). Results from an extended (1994-2007) selection study for resilience to GIN parasite challenge in New Zealand Romney sheep have reported increased average 'age-at-first-drench' in elite resilient lines by 23.6 days relative to their control counterparts (Morris et al., 2010) which was accompanied by a 4.5 kg increase in 6-month live weight. In the same study however, the authors reported that resilient ewe lambs under low chemical treatment struggled to maintain live weight in autumn (March) when levels of nematode challenge were the highest. They suggested that highly productive resilient animals selected under minimum anthelmintics can get overwhelmed in conditions where infections are high to the point that they may contribute to greater losses associated with parasitism and the need to treat them. Similarly, Greer (2008) also suggested that there is little or no evidence from the past few hundred years that relatively intense selection for increased productivity leads to increased ability to cope with a parasite challenge in sheep particularly in the temperate regions.

Even in results from the few farmlet studies, there are inconsistencies regarding the actual realized production benefits relative to the predictions. In Perendale sheep, Leathwick et al. (1998) reported 1.7 kg increase in lambs' live weight compared with susceptible counterparts while fleece weight for hogget and ewes were increased by 5% and 8% respectively, being associated with the benefit of reduced pasture contamination. In another study involving Romney sheep, the authors reported no differences in loss of production resulting from parasite infection between resistant and susceptible selection line animals when grazed separately (Bisset et al., 1997a)

Table 2:2 Least-square mean live weight and weight gains (kg) of lambs in each faecal egg count selection line, by year of birth grouping (Compiled by Morrie et al 2000)

			High FEC		Low FEC	H-L	
Traits	**Years**	**No. of lambs**	**(H)**	**Control**	**(L)**	**Mean**	**s.e.**
Weaning weight	1985 to 1992	2108	19.1	18.2	19.0	0.1	0.2
	1993 to 1997	1725	18.9	19.3	19.1	-0.2	0.2
Post-weaning gain	1985 to 1992	1900	11.0	10.7	10.7	0.3	0.2
	1993 to 1997	1358	9.9	8.7	9.0	0.9***	0.2
Autumn weight	1985 to 1992	1908	30.3	28.9	29.9	0.4	0.2
	1993 to 1997	1360	29.0	27.8	28.0	1.0 ***	0.2
Yearling weight	1987, 1994, to 1996	426	42.3	42.6	40.6	1.7	0.6

2.5 Summary

Nutritional costs associated with the acquisition phase of immune activation during nematode infections has been suggested as the cause of production losses often observed in ruminant animals. Although breeds differ in their level of immunity, young animals are particularly most impacted with infection because of the need for both increased growth and immune development which competes for the same nutrient resources. The effects of parasitism and resulting increased nutrient demands can be made worse if the nutrtitional state of the animals is low, partuclarly in metabolizable protein, which is in high demand. Further, animals differ in both the recognition and magnitude of antigenic stimulus resulting from an infection. Thus, although all animals may eventually be expected to develop immunity to infection, the difference in the timing when this occurs is not yet clear. This is particularly the case with regards to Romney animals that have been selected for the divergence in the way they respond to nematode challenge. Resistant line animals typically exhibit greater immune response compared with resilient or susceptible control animals, but they also appear to have a reduced growth potential when these selection-line animals graze together. If the degree of resistance is believed to enhance the fitness of an individual, in practice selection for resistance should result in animals that are more productive than susceptible or resilient animals that, in some ecxeptional cases, may require minimal anthelmintic treatments to maintain productivity under challenge. However, a question of why most resistant breeds and or selection lines are smaller when compared with resilient and or highly productive but susceptible ones still exists. Further, the role of the degree of maturity may have in the initiation of immune development has been suggested and requires further exploration. It remains to be determined if physiological maturity may help explain the observed differences in resistance to internal parasites between Romney selection lines when compared at the same chronological age.

Various models have predicted greater epidemiological benefits of selecting for increased nematode resistance, with suggested reductions in pasture larvae contaminations. Some models predicted associated gains in live weight and fleece weight by selecting for resistance. However, it is not clear how much of the predicted benefits can actually be realised in field studies when animals with differing levels of resistance are grazed separately in set stocked farmlets. Studies involving production comparisons between resistant and resilient animals have reported poorer growth rates in resistant animals, which partly may have resulted because such comparisons have often been made under conditions where both selection line animals were grazed together, which may have nulified the expected production benefits from resistance. Thus, it remains to be determined if the predicted epidemiological benefts of reduced pasture contamination from selecting for nematode resistance results in actual realisable growth advantage over selection for resilience.

What is also not clear, is whether there is potential for breakdown in resilience at a certain pasture larvae threshold levels, and how this may be attributed by the presence of different related species. Does either resistant or resilient selection favour specific nematode species to flourish? Understanding the resulting parasite dynamics may help in determining the relative contribution of species of parasite to the production losses.

Chapter 3

Variation in timing of immune development to gastro-intestinal nematode parasite infection between Romney selection lines

3.1 Introduction

Selection of sheep for either resistance or resilience to gastrointestinal nematode (GIN) parasites are two tools available to reduce the effects of parasites on animal productivity, health and welfare which, when used in combination with other measures, may substantially reduce the reliance on anthelmintics (Bishop et al., 2004). On the other hand, breeding sheep for increased productivity leads to animals that are largely dependent on anthelmintics for acceptable production (Eady et al., 1998; McEwan et al., 1997; McEwan et al., 1992; Morris et al., 2010) particularly if there is limited or no concurrent selection pressure for parasite resistance. Contrary to this, animals selected for their enhanced resistance to parasites are often observed to have a lesser growth potential (Baker & Gray, 2004; Bisset et al., 2001; Eady et al., 1998; Gamble & Zajac, 1992; Good et al., 2006; Gruner et al., 2003; Matika et al., 2003; Morris et al., 2005; Mugambi et al., 1997) with nutrient resource allocation theory suggesting priority may be given to immune functions over growth (Coop & Kyriazakis, 1999; Coop & Kyriazakis, 2001). Such animals, however, are less attractive to producers particularly in temperate regions where the predominant parasite species have a relatively low level of pathogenecity.

In New Zealand, lines of Romney sheep selected for resistance or resilience to GIN parasites have been established and maintained over several decades resulting in two lines of animals with considerable differences in immunological and parasitological profiles. Resistant-line animals' FEC have reduced by 85% or more (Bisset et al., 1991b; Morris et al., 1995b) whereas an increase in the average age at first drench by 23.6 days has been reported in resilient line animals (Bisset et al., 1994; Morris et al., 2010). While it is expected for resistant Romney lines to exhibit greater immunological capacity compared with resilient or susceptible animals (Bisset et al., 1996b; Douch et al., 1995; Douch et al., 1994; Green et al., 1999; Shaw et al., 1999; Shaw et al., 2012), the difference in timing and the magnitude of immunological responses in these selection lines is less clear. The timing of which animals develop immune capacity to GINs has been suggested to be associated with metabolic age, rather than chronological age (Greer & Hamie, 2016). Further, considerable variations exist in time taken to mature between different breeds and even among individuals of the same breed (Taylor, 1965; Taylor, 1985). Thus, it is possible the development of immunity in resistant line may reflect an earlier development of maturity.

Although the acquisition of immunity is known to be nutritionally costly to the host animal, studies by Greer et al. (2018) indicated a comparable cost between Romney lines when both line-animals are given similar levels of infection with 80 *T. colubriformis* larvae per kilogram live weight and allowed the opportunity to acquire immunity. It is therefore postulated that the difference in performance observed in these selection lines (de Lautour & de Lautour, 2010; Morris et al., 2000; Morris et al., 1997; Wheeler et al., 2008) may reflect a temporal differences in the timing of immunity, i.e., resistant animals may be more relatively mature at same chronological age. A better understanding of the timing of immune development in relation to metabolic (physiological) age may help explain the differences in performance attributes that have been reported in these Romney selection lines. The objective of the study was to test the hypothesis that development of immunity, in part, may be a function of animal maturity through assessing the variations in timing of development of immunity and immunological responses in resistant and resilient Romney selection lines exposed to mixed natural GIN parasite infection.

3.2 Materials and methods

3.2.1 Experimental site and animal management

All proceedures were carried out with approval from, and in accordance with the Lincoln University Animal Ethics Committee, application number LUAEC #588.

3.2.2 Animals and sampling

Variation in the timing of development of immunity and immunological responses was assessed in two lines of Romney sheep that had undergone greater than 16 generations of selection either for resistance or resilience to GIN parasites. These lines were established by AgResearch in 1979 (Bisset et al., 1996; Morris et al., 2000) and were actively selected within line initially based on faecal egg count (FEC) and ability to maintain performance without anthelmintic. In 2008 they were transferred to Lincoln University from which point they were maintained without anthelmintic treatment and replacement ewe and ram lambs were randomly selected within each line each year. Twenty-six male and 27 female lambs, which comprised of the total number of the animals born in 2014, were grazed together on paddocks containing predominantly ryegrass pasture to allow for natural infection with mixed parasite species. Of the 53 lambs, 23 were from the resistant (10 rams and 13 ewes) and 30 were resilient (16 rams and 14 ewes) selection lines. From weaning, at a mean 92 days-of-age, animals were sampled approximately every 10 days until 351 days-of-age. No anthelmintic treatment was administered at any time. At each sampling time, faecal samples were collected from the rectum of each lamb immediately upon yarding for the determination of the concentration of nematode eggs in the faeces using a modification of the McMaster method by floatation in saturated sodium chloride solution as described by MAFF (1984). Briefly, 1.7 g of faeces were placed in a jar with 5ml of water, and soaked overnight to soften. The following day, 46 ml of saturated sodium chloride (NaCl) was added and the sample mixed for 25 seconds with an electrical stirrer, untill the faecal pellet was completely broken up. A pasteur pippette was used to fill both chambers on a moistened McMaster slide with the faecal suspension taken immediately from the middle of the jar. Any eggs present on the slide, float to the surface of the salt solution and these were counted under a microscope and totals multiplied by 100 to give the number of eggs per gram of faeces (epg). Saliva samples were taken using mouth swabs that were then centrifuged at 1200 x g for 2 minutes and clean saliva was stored at -20 ^{o}C untill analysis. Animals were then fasted without access to feed or water for 16 hours before the recording of fasted live weights after which they were returned to grazing. Liveweights were recorded with the use of electronic identification tags (Allflex New Zealand) and an Aleis tag reader connected to a semi-automated Prattley autodrafter with a sensitivity of 0.2 kg.

3.2.3 Grazing management

Romney lambs were born and reared on pasture at the Lincoln University Ashley Dene Research Farm and weaned at mean of 92 days-of-age. At a mean of 124 days-of-age lambs were moved to the Lincoln University "LincolnSheep" Research Farm where they grazed on irrigated pastures and were exposed to continued natural larval challenge comprising mixed-species nematode parasites untill 351 days-of-age. Animals had access to *ad libitum* pasture until 280 days-of-age at which point pasture was resticted to a winter grazing allocation. At all times resistant and resilient lambs were grazed together to ensure similar parasite exposure and a similar nutritional environment. From day 215, female and male lambs were seperated and grazed separately and a vasectimised ram fitted with a mating harness was introduced into the female cohorts. The date of the first presence of mating marks on ewe lambs (n=27) were recorded at subsequent sampling times as an indicator of oestrus to indicate the development of sexual maturity.

3.2.4 Estimated Relative Maturity

The expected mature weight of resistant and resilient Romney female lambs were estimated based on the weights of individual animals when mated by a vasectomized ram which represents 60% of their mature weight as suggested by Taylor (1985). The relative maturity of all individual females lambs were calculated as a proportion of current weight at each sampling point divided by its expected mature weight. Estimates of relative maturity were not determined for male lambs

3.2.5 ELISA

Infective larvae (L3) were collected following the culture of faeces from separate sheep mono-specifically infected with either *Teladorsagia circumcincta* or *Trichostrongylus colubriformis*. Approximately 1 million L3 larvae contained in 100 ml were centrifuged at 10,000 x g for 2-minutes with the supernatant discarded, leaving a larval pellet which was frozen and thawed four times before homogenisation using a Mini Beadbeater™ (Biospec). Tubes containing the larvae and steel ball bearings were shaken multiple (15) times with each shaking lasting for 30 seconds followed by a minimum of 2-minutes in an ice bath to avoid protein denaturation. Following centrifugation for 2 minutes at 10,000 x g the L3 antigen supernatant was removed and the protein concentration determined using BCA Pierce Protein Assay Kit.

3.2.6 ELISA analysis

Saliva samples were analysed for antibodies to L3-specific *T. colubriformis* and *T. circumcincta* larvae using an enzyme linked immunosorbant assay (ELISA) similar to that described by Douch et al. (1994). For both *T. colubriformis* and *T. circumcincta,* 50 µl of L3 antigen/well at 2 µg/ml in coating buffer were incubated at 4 ^{o}C overnight in 96-well plates.

The ELISA plates were washed 5 times with dilution buffer containing 0.1% (w/v) Tween 20 (W-T20), ten blocked using 200 µl/well of 10 mM-phosphate buffer at pH 7.2 containing 0.5% Tween-20 and 5% bovine skim milk powder that was incubated for 2 h at room temperature. Plates were then washed 5 times with wash buffer. Diluted saliva (1:10 for IgA and 1:100 for IgG) was added to ELISA plates at 50 µl /well, incubated for 2 h and then plates were washed 5 times with wash buffer. Rabbit anti-sheep IgG (Pierce immunopure Antibodies, cat #31480, lot #GI959969) at a dilution of 1:4,000 and IgA conjugated with horseradish peroxidase (Pierce immunopure Antibodies, Lot#A-130-108P-35 Bethly Laboratories inc., USA), diluted 1:2000 with ELISA buffer, was added to each well (100 µl) and incubated for 1 h at room temperature. Plates were washed 5 times with wash buffer. To develop colour, 100 µl/well of 0.05 M phosphate-citrate buffer which was adjusted to pH 5.0 with 0.02% of 30% H_2O_2 added and containing 100 µg of tetramethyl benzidine dihydrochloride (Sigma Aldrich, U.S.A) was incubated for 40 minutes at room temperature. The reaction was stopped by adding 100 µl/well of 1.25 M H_2SO_4. and the optical density (OD) at 450nm read using ELISA plate reader (Multiscan Go, 1510-01462C, Thermofisher Scientific Finland). Results were adjusted according to a standard positive serum sample present on each plate and expressed as the mean optical density (OD) value of duplicate wells.

3.2.7 Animal variations within selection lines

Within each selection line, individual animals were re-classified into four subclasses based on their capacity to either develop immunity, grow above average or a combination of the two, namely: 1) immune and growth (+/+), 2) not immune and growth (-/+), 3) immune with no growth (+/-)and 4) not immune and no growth (-/-). The four traits used in classification were Live-weight (LW), faecal egg count (FEC), immunoglobulin IgG and IgA responses. For immunity, IgG, IgA and FEC were used as indicator traits. For all the traits, a weighted mean was used, and an animal needed to have a mean IgG and IgA above the weighted mean whereas for FEC animals needed to have less than the weighted mean, respectively. An animal was deemed as + for immunity if it had a combination of at least two of the three indicators traits. For classification of + growth, animals needed to have combine LWG greater than the mean.

3.2.8 Animal variations disregarding initial selection lines

All animals were then pooled together disregarding their initial line of selection and sex and regrouped in subclasses based on similarities in the characteristics between similar subgroups between selection lines as was described in section 3.2.7 above. The resulting subgroups were subjected to statistical analysis

3.2.9 Statistical analysis

Statistical analysis was performed using Genstat statistical package 18th edition (Version 18.1, VSN International Ltd). Faecal egg counts were log10 (n+100) transformed prior to analysis with results reported as back-transformed means. Faecal egg counts, IgG and IgA, liveweight and relative maturity were subjected to sequential comparison for antedepence structures prior to analysis as repeated meaures using a Restricted Maximum Likelihood Model (REML) with time, selection line and sex included as factors and animal as a random factor.

To avoid arbitraries in choosing single overall IgG threshold at which animals would be deemed to have developed immunity, a Receiver Operating Characteristic (ROC) analysis was used to determine the cut-off point and / or threshold. At any sampling point and for each individual animal, FEC < 200 epg were assigned as true immunity and coded as 1 whereas those ≥200 epg were regarded as false immunity and were coded as 0. These codes were then run paired with the actual corresponding IgG OD-values at each specific sampling time. The resulting correlated values were used to calculate the quality of the test and then plotted with the area under curve giving the probability of having a positive diagnosis test. With this analysis, an optimum IgG threshold of 1-OD was determined which was associated with FEC<200 epg.

3.3 Results

3.3.1 Live weight (LW)

Mean fasted live weight (kg) for female and male resistant and resilient-line animals is given in Figures 3.1. At weaning (92 days-of-age), live weight showed a selection line by sex interaction (P=0.007) reflecting heavier weights in resilient males than their female counterparts whereas male and female resistant animals were similar, *viz.* 24.9 ± 0.46, 20.8 ± 0.68, 17.7 ± 1.37 and 18.3 ± 0.93 kg for resilient males, resilient females, resistant males and resistant females respectively. Overall, mean fasted live weight showed a selection line by time interaction (P<0.001) which reflected consistently greater LW in resilient-line animals groups, which peaked at day 280 before declining and being similar for all groups from day 330. Sex had an effect (P=0.052) reflecting heavier males than females *viz.* (29.02 ± 0.67 *c.f.* 27.24 ± 0.73 kg) but there was no interaction between selection line and sex (P=0.155).

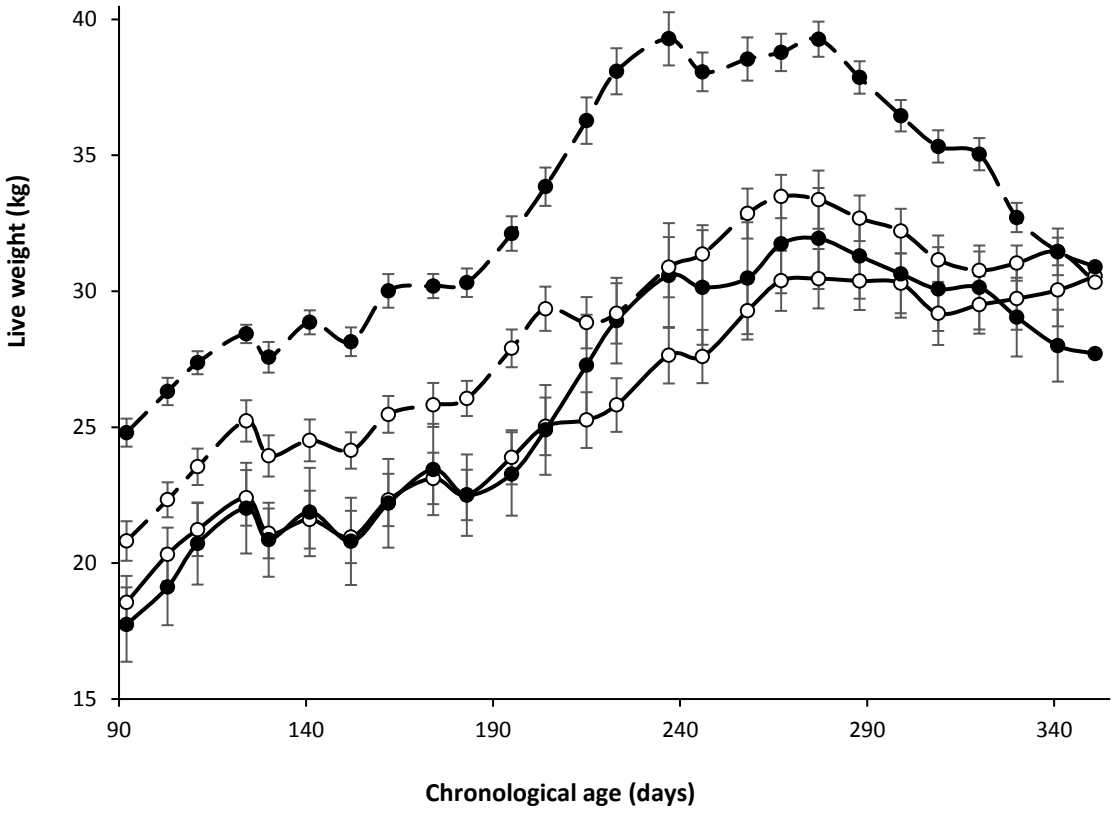


Figure 3:1 Mean live weight (kg) of resistant (solid line), and resilient (dashed line), males (close circles) and females (open circles) Romney sheep exposed to a natural mixed-species GIN parasite infection

3.3.2 Live weight gain (LWG)

Mean cumulative live weight gain (kg) for female and male resistant and resilient-line animals are given in Figures 3.2. Overall, there were interactions between selection line and time (P<0.001) and between sex and time (P<0.001) that reflected liveweight gains between days 190 and 260 that were greater for males than females and greater for resilient than resistant animals followed by a greater decline in live weight gain in resilient and in males from day 290

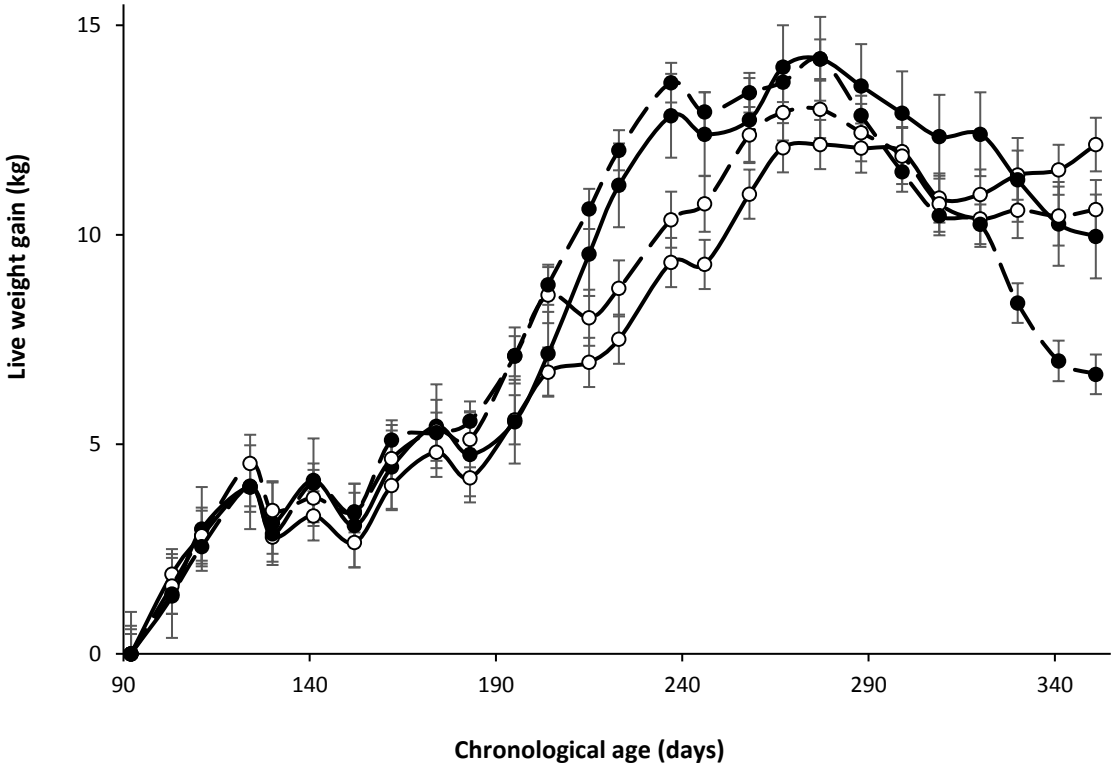


Figure 3:2 Mean live weight gain (kg) of resistant (solid line), and resilient (dashed line), males (close circles) and females (open circles) Romney sheep exposed to a natural mixed-species GIN parasite infection

3.3.3 Slaughter weights

Cumulative percentage (%) of male and female animals from resistant and resilient Romney selection lines that reached a slaughter weight of 35 kg are given in Figure 3.3. Eighty one percent of resilient males reached the slaughter weight by day 215 and 100% by day 277 whereas 20% of resistant male lambs attained this weight by day 215 and a maximum of only 30% by day 237. In female lambs, only 35.7% and 23.1% of resilient and resistant animals reached the slaughter weight by days, 277 and 299, respectively.

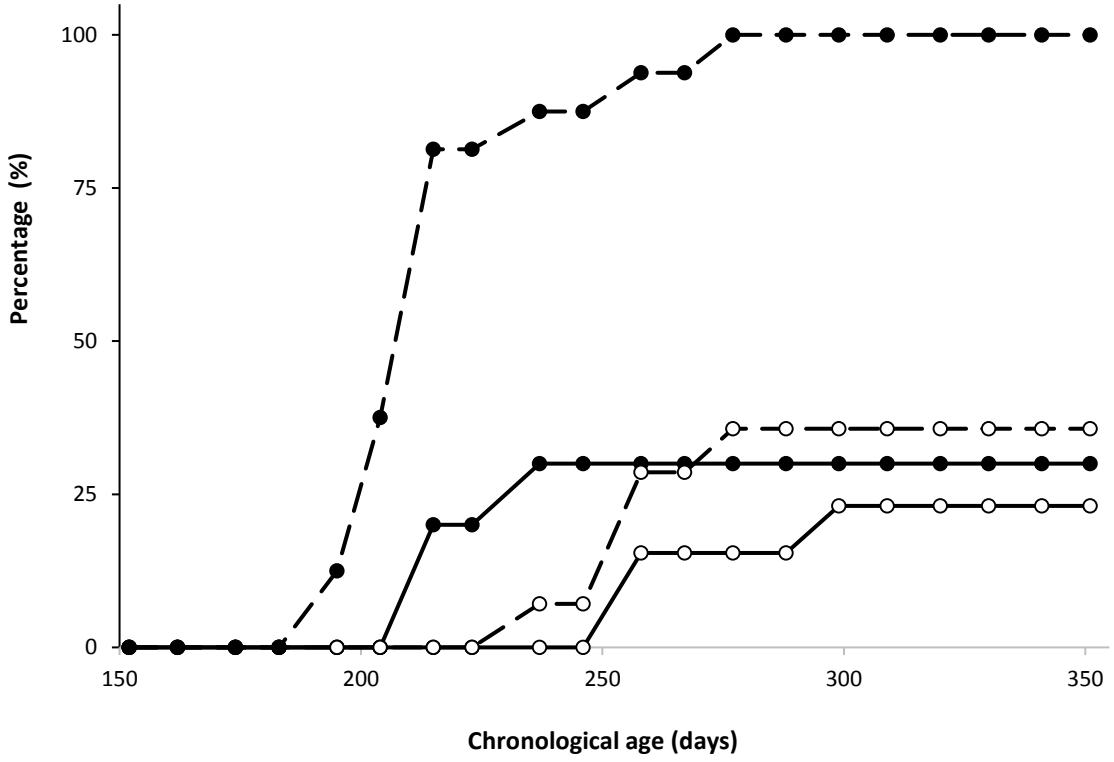


Figure 3:3 Cumulative percentage (%) of resistant (solid line), and resilient (dashed line), males (close circles) and females (open circles) Romney sheep exposed to a natural mixed-species GIN parasite infection

3.3.4 Physiological maturity

Sexual maturity

Cumulative percentage (%) of resistant and resilient female lambs with mating marks are presented in Figure 3.4. Overall, the mean age that mating marks were recorded in resistant-ewe lambs was earlier than their resilient counterparts, *viz.* 263.5 ± 3.7 days and 274.4 ± 3.4 days, respectively (P=0.048). Mean liveweight at the first record of mating marks was less for resistant than resilient line females *viz.* 30.4 kg *c.f.* 35.0 kg respectively (P=0.019).

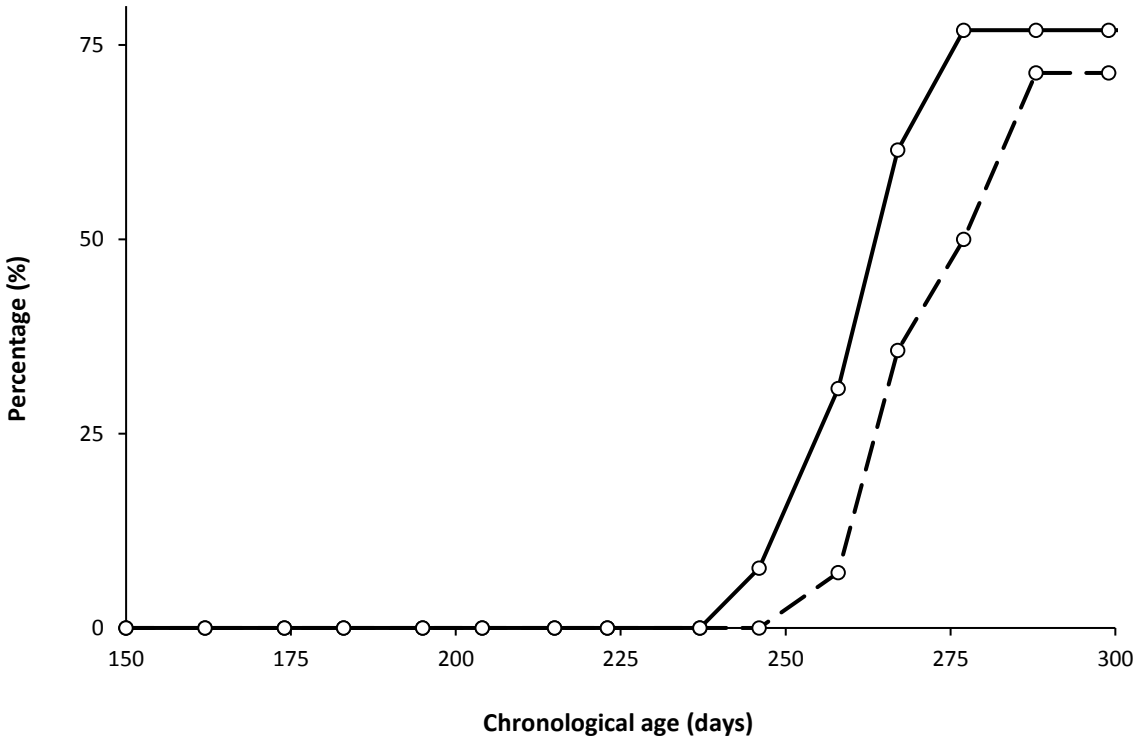


Figure 3:4 Cumulative percentage (%) of resistant female (solid line, open circle) and resilient female (dashed line, open circle) lambs that reached sexual maturity as indicated by indirect mating marks from vasectomised ram fitted with harness

3.3.5 Relative maturity (RM)

Mean estimated relative maturity (proportion of current weight divided by its expected mature weight) for resistant and resilient females is given in Figure 3.5. Overall, relative maturity showed a significant interaction between selection line and time (P<0.001) which reflected similar low initial values which increased with time in both lines but being greater for resistant than resilient animals between 250 and 300 days-of-age. However, the slope of the lines was similar in both selection lines *viz.* 0.0020 ± 0.0005 *c.f.* 0.0010 ± 0.0005 for resistant and resilient females respectively (P=0.182).

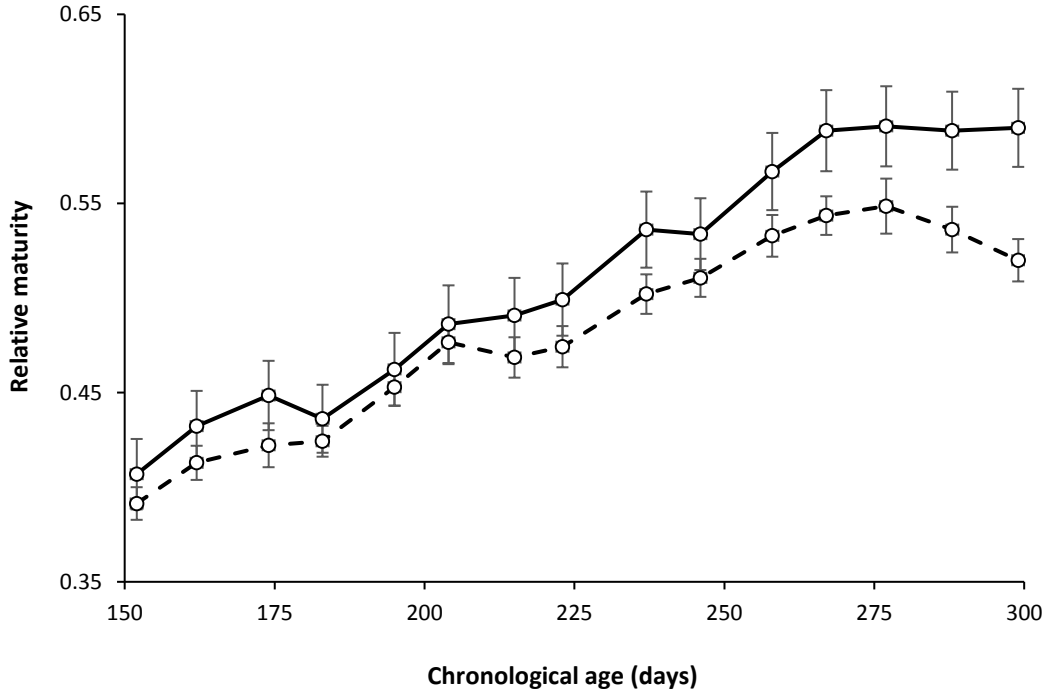


Figure 3:5 Relative maturity in resistant female lamb (solid line, open circles) and resilient female lambs (dashed line, open circles) with time

3.3.6 Faecal egg count (FEC)

Mean back-transformed FEC (epg) of female and male Romney lambs from selection lines for resistance or resilience are given in Figure 3.6. Overall, FEC showed a selection line by time interaction (P<0.001) that reflected FEC being similar between lines and initially low and FEC typically remained at less than 200 epg for resistant line animals whereas resilient line animals gradually increased to a peak of 1400 epg at day 230 for females and 1800 epg for males at day 280 before declining to less than 500 epg by day 300. There was no effect of sex (P=0.655) and no interaction between selection line and sex (P=0.906)

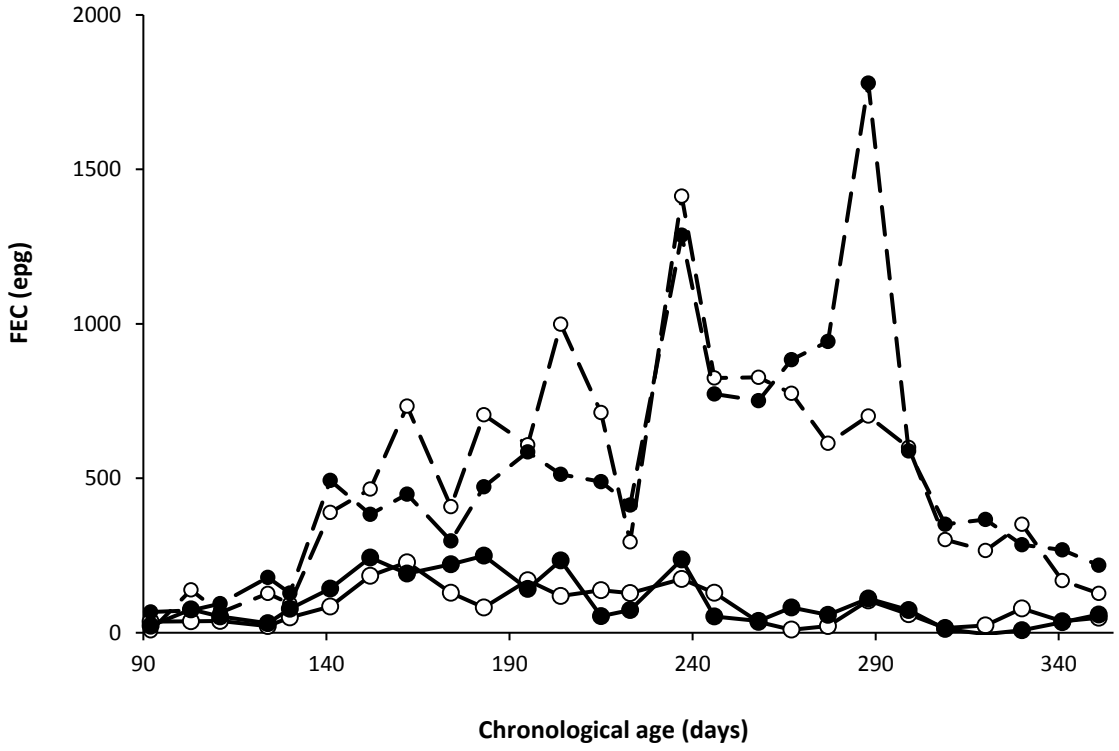


Figure 3:6 Mean back-transformed FEC (epg) of resistant (solid line), and resilient (dashed line), males (close circles) and females (open circles) Romney sheep exposed to natural mixed-species GIN parasite infection

3.3.7 Immunoglobulin G (IgG)

Mean serum L3 *T. colubriformis*-specific IgG and *T. circumcincta* L3-specific IgG profiles are given in Figures 3.7 and 3.8 respectively. *T. colubriformis* L3-specific IgG showed a selection line by time interaction ($P<0.001$) reflecting low initial values followed by an increase in all groups with time to similar maximum values at day 330 which occurred earlier in resistant than resilient-line animals. There was no effect of sex on IgG responses ($P=0.159$) and no interaction between selection line and sex ($P=0.522$) as within selection line, both male and females appeared to show similar patterns in their IgG response.

For *T. circumcincta* L3-specific, IgG showed a selection line by time interaction ($P=0.016$) reflecting low initial IgG responses which increased with time in all groups up to day 330 but being greater in resistant than resilient counterparts. In addition, the interaction between sex and time was also significant which reflected greater increase with time in resistant females than resistant males between days 215 and 250 and then a subsequent greater response in males than female resistant animals between days 320 and 340 whereas IgG for resilient males and females remained similar. However, there was no interaction between selection line and sex ($P=0.684$).

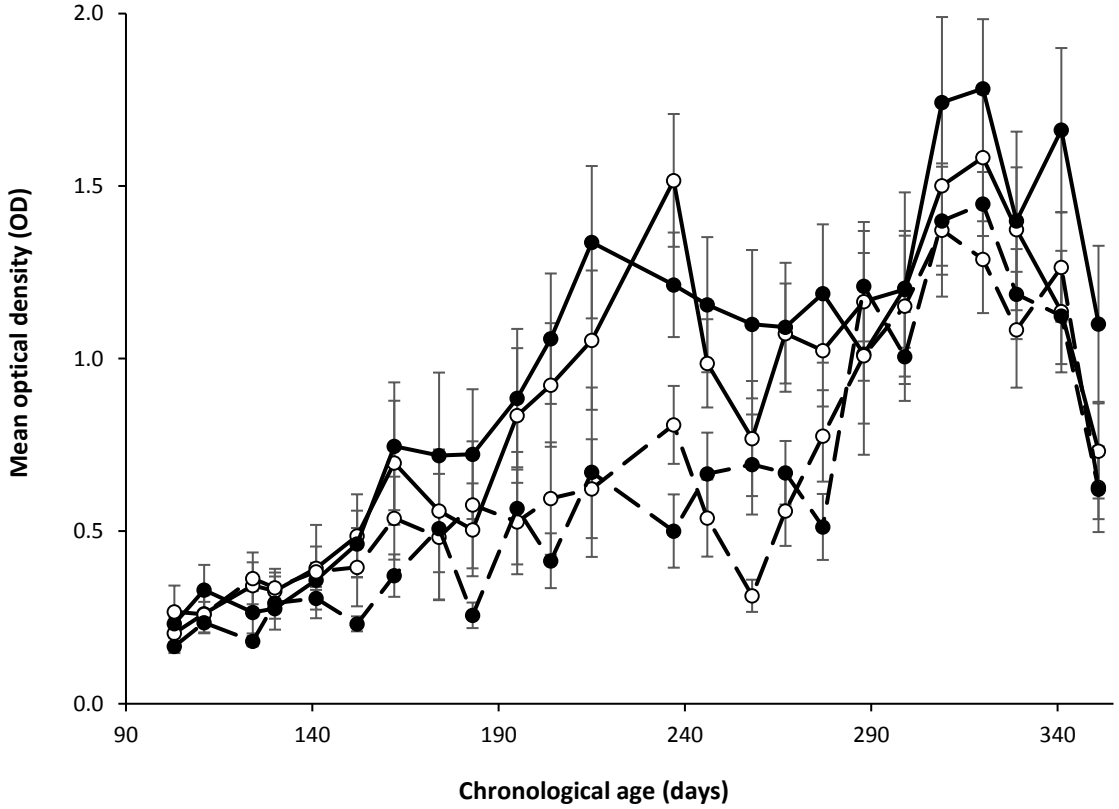


Figure 3:7 Mean Optical density (OD) for IgG responses to *T. colubriformis* L3-specific antigen of resistant (solid line), and resilient (dashed line), males (close circles) and females (open circles) Romney sheep exposed to natural mixed-species GIN parasite infection

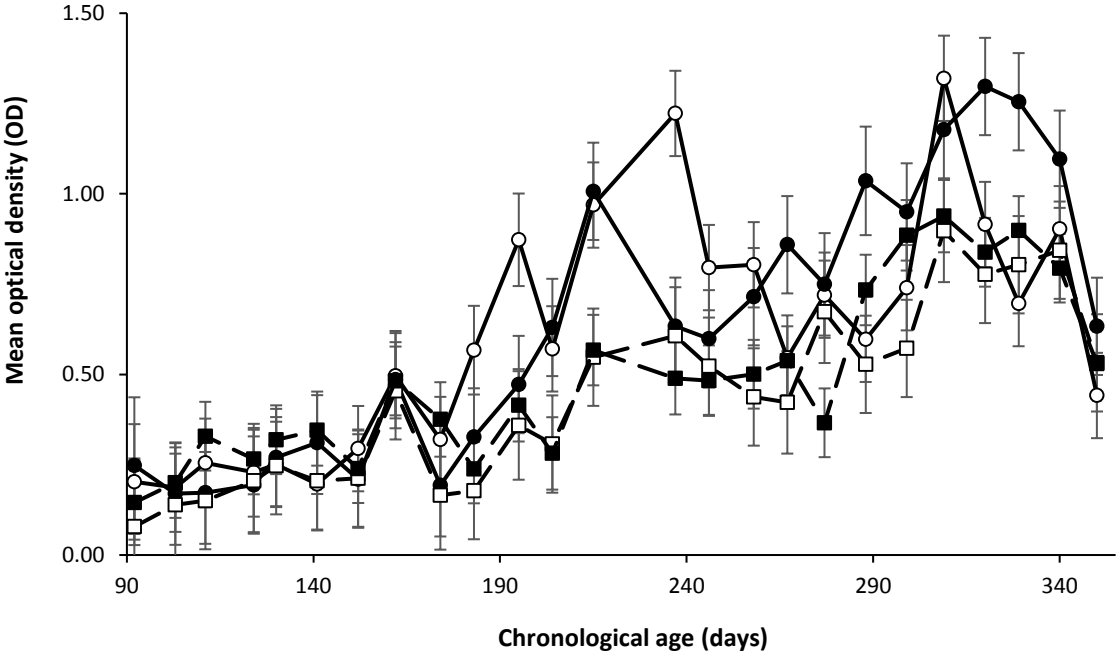


Figure 3:8 Mean Optical density (OD) for IgG responses to *T. circumcincta* L3-specific antigen of resistant (solid line), and resilient (dashed line), males (close circles) and females (open circles) Romney sheep exposed to natural mixed-species GIN parasite infection

3.3.8 Immunoglobulin A (IgA)

Mean serum L3 *T. colubriformis* L3-specific and *T. circumcincta* L3-specific IgA profiles for male and females from Romney selection lines for resistance and resilience are given in Figures 3.9 and 3.10 respectively. Overall *T. colubriformis* L3-specific immunoglobulin A (IgA) showed selection-line by time interaction (P=0.037) reflecting low initial levels followed by a greater rise in resistant between days 190-230, then peaking at day 300. There was a tendency for greater IgA levels in females than males, *viz.* 0.627 ± 0.025 *c.f.* 0.571 ± 0.024, respectively, (P=0.065) but there was no interaction between selection-line and sex (P=0.207) nor between sex and time (P=0.404).

T. circumcincta, L3-specific IgA showed a selection line by time interaction (P=0.045) which reflected similar initial low values which increased in all groups between days 200 and 250 but the rate of increase was greater in resistant than resilient counterparts and then remained relatively similar in pattern in all selection lines. Sex had no effect (P=0.780) and there were no interactions either between sex and time (P=0.971) or between sex and selection line (P=0.137).

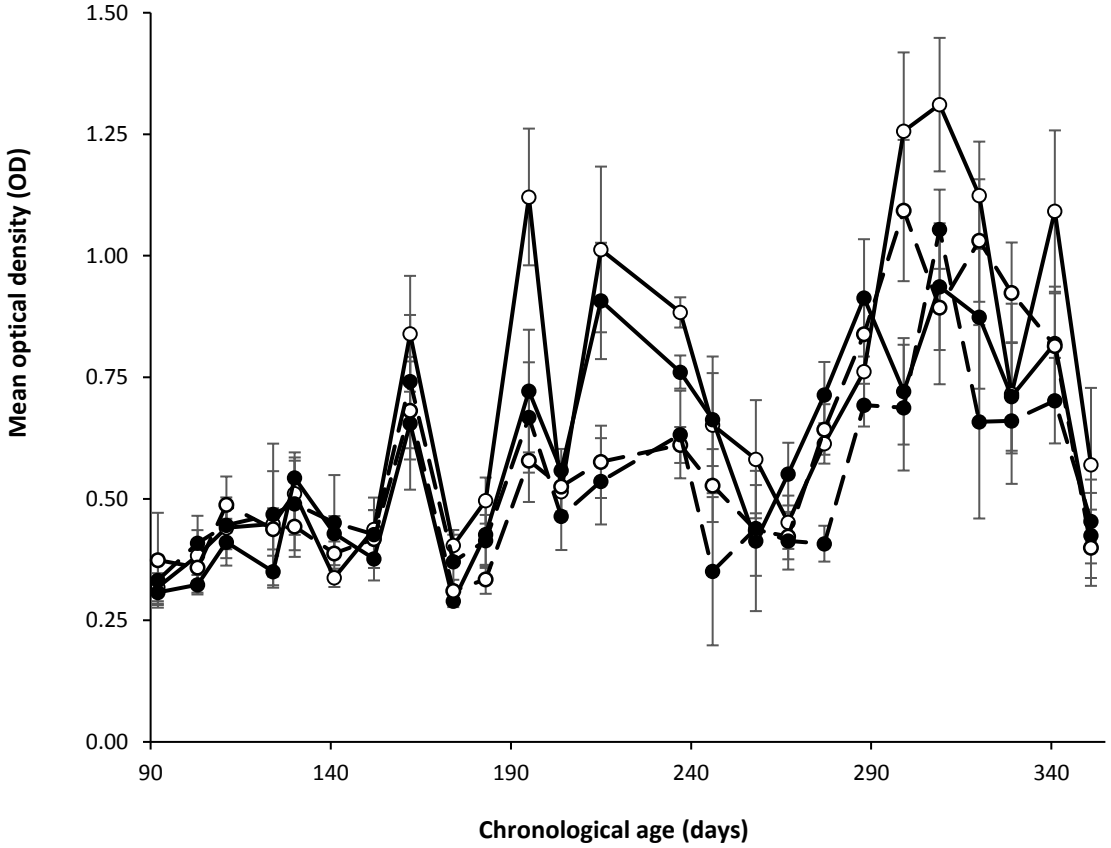


Figure 3:9 Mean Optical density (OD) for IgA responses to *T. colubriformis* L3-specific antigen of resistant (solid line), and resilient (dashed line), males (close circles) and females (open circles) Romney sheep exposed to natural mixed-species GIN parasite infection

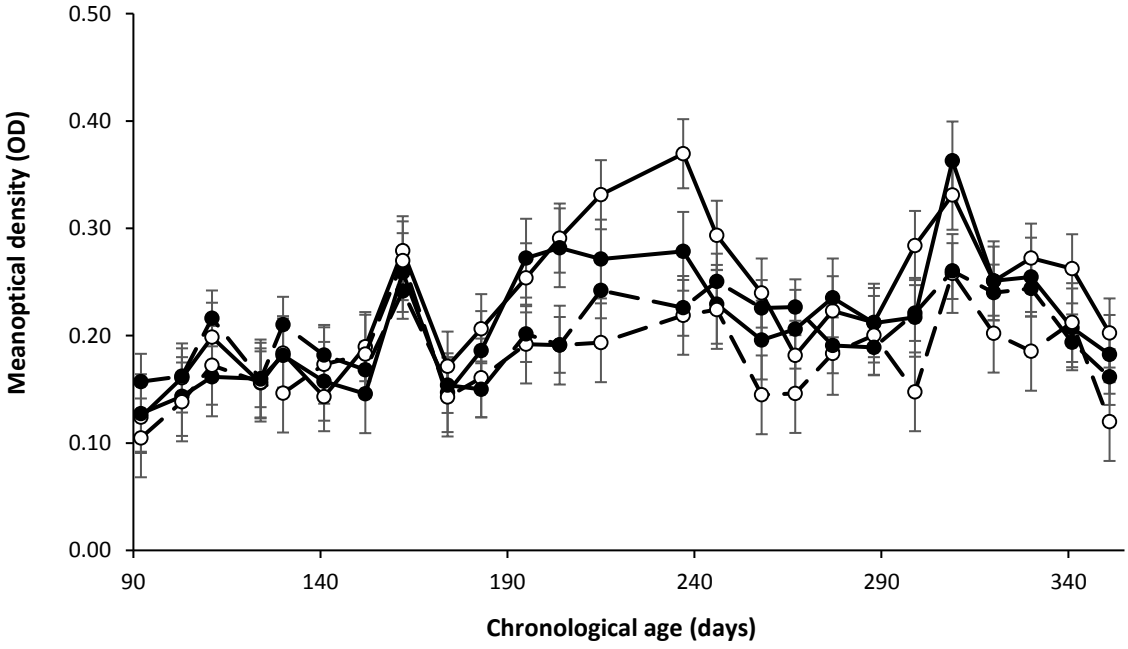


Figure 3:10 Mean Optical density (OD) for IgA responses to *T. circumcincta* L3-specific antigen of resistant (solid line), and resilient (dashed line), males (close circles) and females (open circles) Romney sheep exposed to natural mixed-species GIN parasite infection

3.3.9 Comparison at similar IgG of 1-OD

Cumulative percentages of female and male lambs reaching a threshold IgG of 1-OD is given in Figure 3.11. The area under area under curve (AUC) from the ROC analysis was 0.628 ± 0.020. Overall, mean age of resistant lambs when they reached the threshold IgG of 1-OD was earlier at 220.6 ± 8.8 days-of-age compared with resilient-line animals which reach this threshold at 263.4 ± 6.9 days-of-age (P<0.001). Within selection line however, the mean age of female and male lambs was similar.

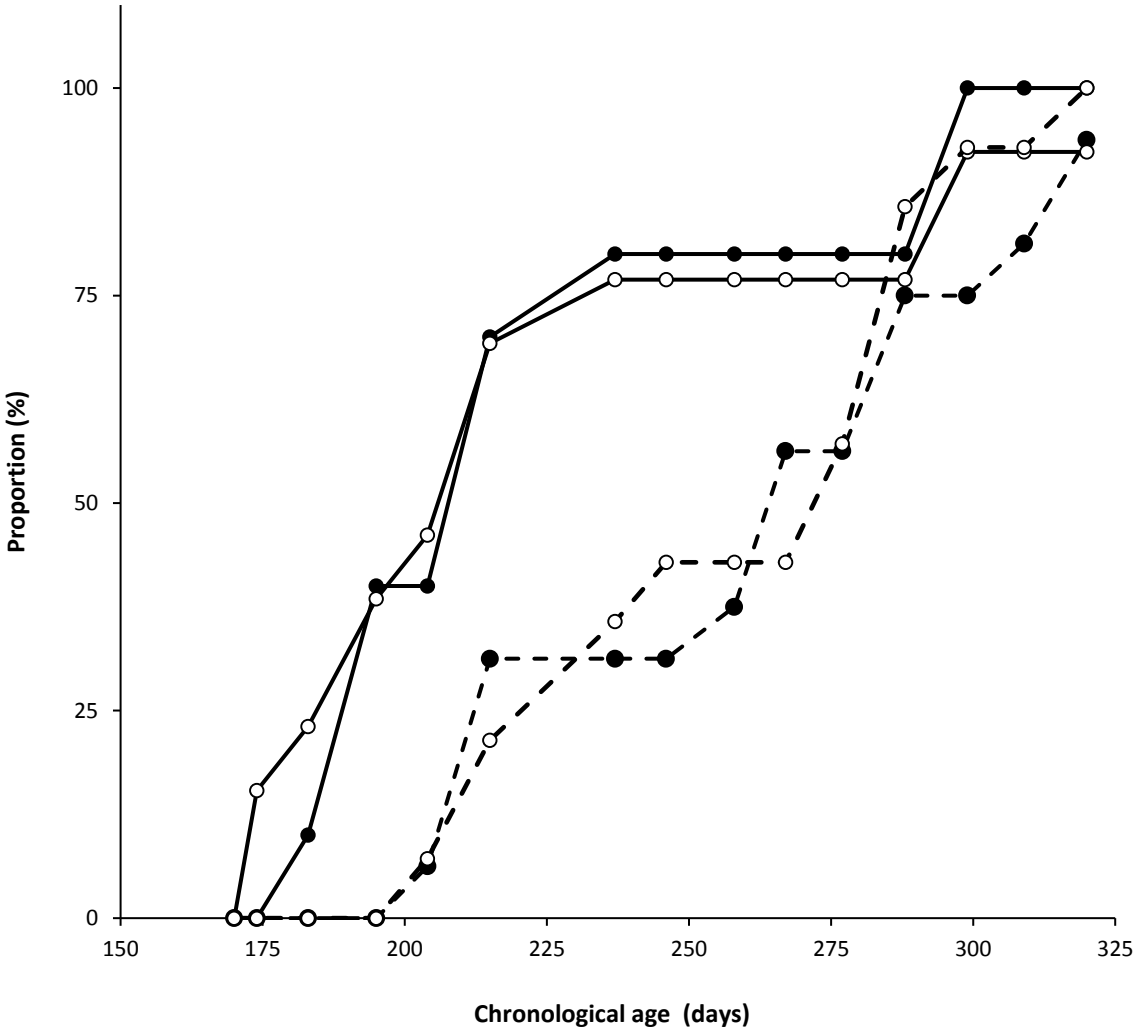


Figure 3:11 Cumulative percentage (%) of resistant female (solid line, open circles), resistant male (solid line, closed circles), resilient female (dashed line, open circles) and resilient male lambs (dashed line, closed circles) reaching the threshold IgG response of 1-OD

3.3.10 Proportional variations within selection lines

Proportion of variations within selection line and sex are given in Table 3.1. Overall, 26.7% of resilient lambs (28.6% females and 25% males), and 30.4% of resistant-line animals (30.8% females and 30% males) were both immune and grew well. All 16 resilient male lambs grew well but 68.8% of these were not immune. Similarly, all 14 female resilient lambs grew well, but 71.4% were not immune. In contrast, 90% of resistant male lambs were immune of which 67% of these did not grow well. Eighty-five percent (84.6%) of the resistant females were immune and only 36.4% of these grew well.

Table 3:1 Proportion of female and male lambs belonging to each of the four subclasses: 1) immune and growth (+/+), 2) not immune and growth (-/+), 3) immune with no growth (+/-) and 4) not immune and no growth (-/-) within resilient and resistant Romney selection lines exposed to mixed- species parasite challenge

Line of Origin	Sex	Sub-class	No of animals	Percentage (%)
Resilient	Females	+/+	4	**28.6**
		-/+	10	**71.4**
		+/-	0	**0.0**
		-/-	0	**0.0**
		Sub-total	***14***	
	Males	+/+	4	**25.0**
		-/+	11	**68.8**
		+/-	0	**0.0**
		-/-	2	**12.5**
		Sub-total	***16***	
		Total	**30**	
Resistant	Females	+/+	4	**30.8**
		-/+	0	**0.0**
		+/-	7	**53.8**
		-/-	2	**15.4**
		Sub-total	***13***	
	Males	+/+	3	**30.0**
		-/+	0	**0.0**
		+/-	6	**60.0**
		-/-	1	**10.0**
		Sub-total	***10***	
		Total	**23**	
		Grand Total	**53**	

Mean FEC (epg) for the four groups of animals within resilient selection lines are given in Figure 3.12 respectively. Animals that grew well but were not immune had the highest levels of nematode FEC with peak of about 4000 epg followed by animals that were not immune and did not grow well.

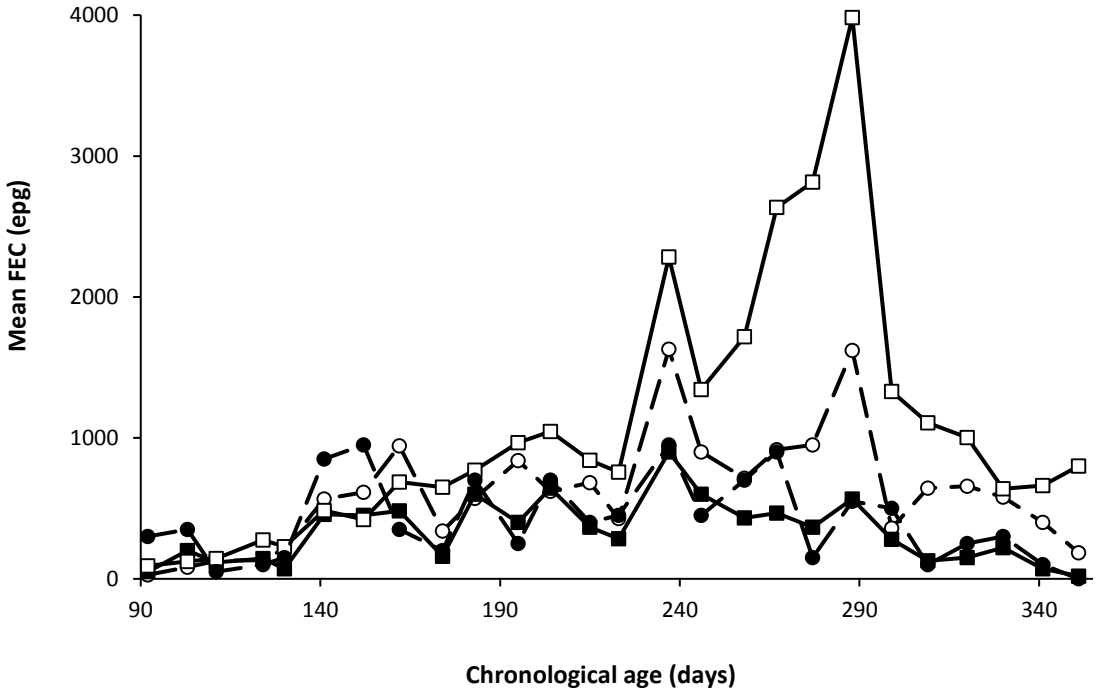


Figure 3:12 Mean FEC (epg) for resilient Romney selection-line animals exposed to natural mixed-species GIN parasite infection grouped into four subclasses: immune and growth (solid line, squares closed), growth but not immune (solid line, squares open), immune but no growth (dashed line, circles closed) and not immune and no growth (dashed line, circles open)

Trends in mean live weight (kg) in resistant and resilient selection line animals are given in Figures 3.13 and 3.14 respectively. Considerable similarities exist in growth potential and trends in live weight between animals from resistant and resilient selection lines that were either immune and grew well or immune but with below average growth. But it was evident from both selection lines that live weight in animals which were immune but did not grow well were consistently lower compared with animals which were both immune and grew well.

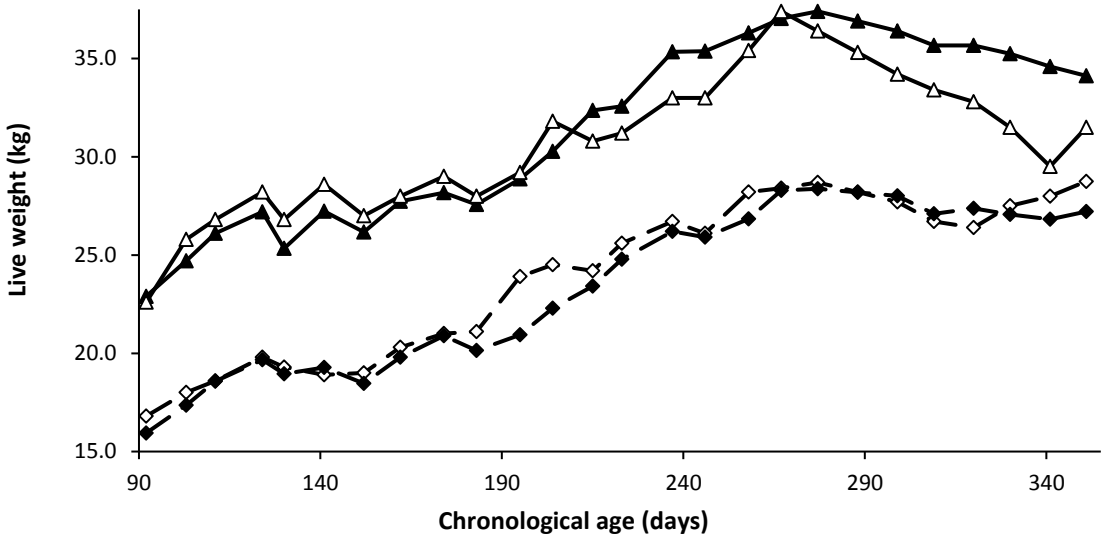


Figure 3:13 Mean live weight (kg) for resistant Romney selection-line animals exposed natural infected with mixed-species GIN parasite and grouped into four subclasses: immune and growth (solid line, triangles closed), growth but not immune (solid line, triangles pen), immune but no growth (dashed line, rhombus closed) and not immune and no growth (dashed line, rhombus closed)

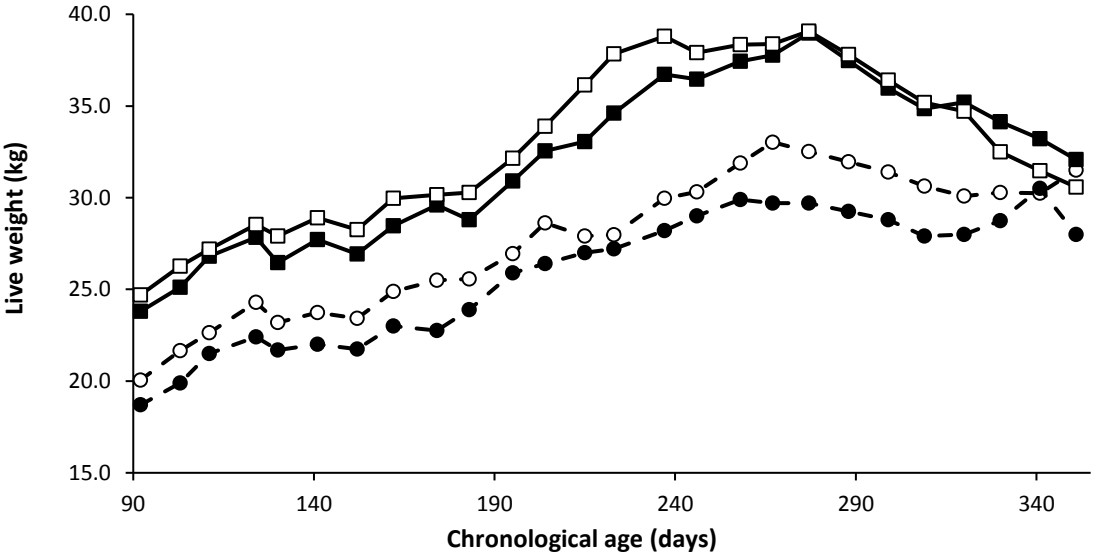


Figure 3:14 Mean live weight (kg) for resilient Romney selection-line animals exposed natural infected with mixed-species GIN parasite and grouped into four subclasses: immune and growth (solid line, squares closed), growth but not immune (solid line, squares open) , immune but no growth (dashed line, circles closed) and not immune and no growth (dashed line, circles open)

Considerable similarities exist in growth potential and profiles in live weight between animals from resistant and resilient selection lines that have either growth and immunity traits or those that are immune but without growth as given in Figure 3.15. But it was evident from both selection lines that live weight trends in animals which are immune but without growth (+/-) were consistently lower compared with animals with both immunity and growth traits (+/+).

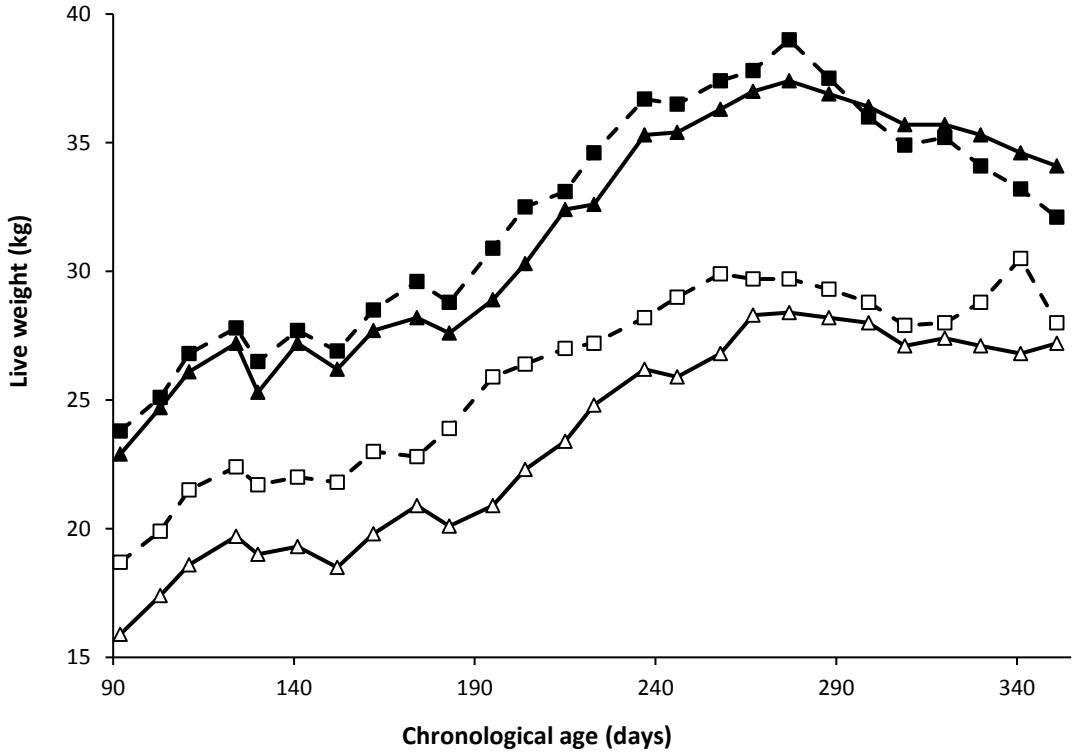


Figure 3:15 Mean live weight (kg) profiles for animals from resistant (solid line, triangles marks) and resilient (dashed line, square marks) selection-line animals that have both immune and growth (+/+; closed) and compared with those that are immune but with no g growth (+/-, open) under mixed-species nematode parasite infection

3.3.11 Animal variations disregarding initial selection lines

A summary of proportions of animals belonging to each of the four subgroups irrespective of their selection-line of origin is given in Table 3.2. Of the 53 lambs, 35 (66%) grew well but only 40% of the 35 were also immune. Contrary, 50% of the 53 lambs were immune but 48% did not grow well. A small portion (9%) represents animals that were neither immune nor grew well.

Table 3:2 Percentages of distribution of Romney sheep lambs exposed to mixed-species parasite challenge that belong: 1) immune and growth, 2) not immune and growth, 3) immune with no growth and 4) not immune and no growth

Subclass	# of Animals	Percentage (%)
Immune + growth	*14*	**26**
Not immune + growth	*21*	***40***
Immune not growth	13	**25**
Not immune not growth	5	**9**
Total	**53**	

Faecal egg count (FEC)

Mean back-transformed FEC (epg) are shown in Figure 3.16. Overall, FEC showed subclass by time interaction ($P<0.001$) reflecting low initial FEC for all subclasses but increasing to reach maximum of >2000 epg for animals that grew well but were not immune to infection while for animals which were immune but did not grow well consistently maintained the least FEC with the other two subclasses being intermediate in FEC profiles.

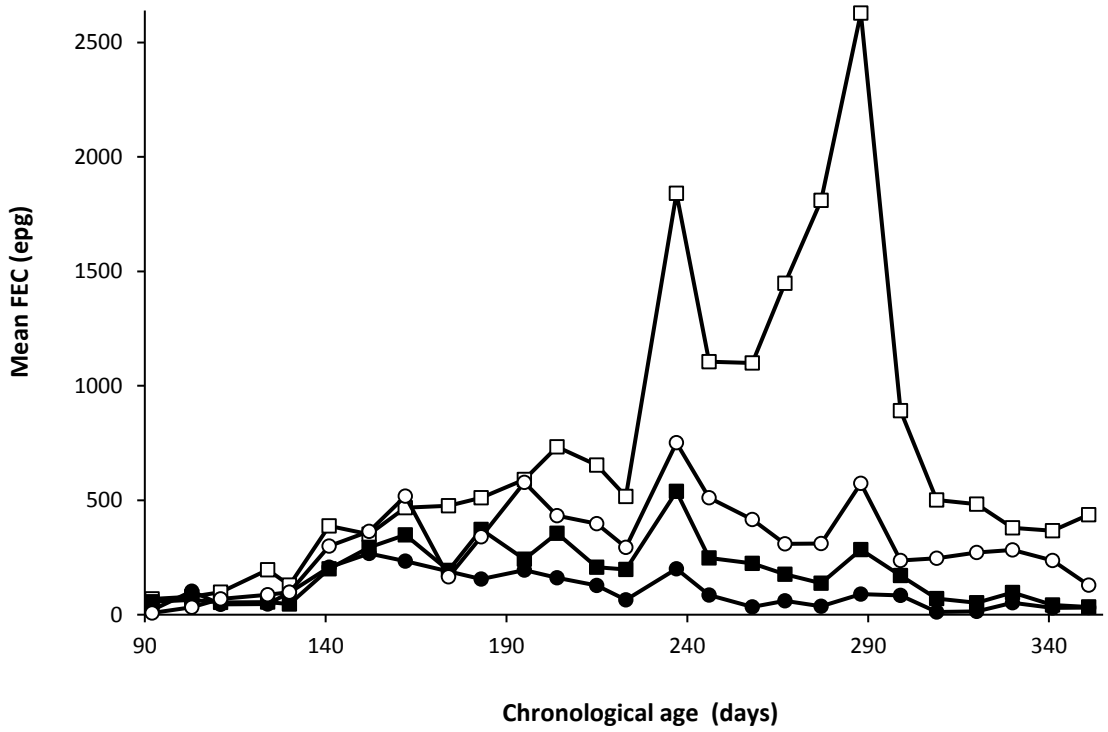


Figure 3:16 Mean back-transformed FEC (epg) for Romney lambs re-grouped into four subclasses: immune and growth (closed squares), growth but not immune (open squares), immune but no growth (closed circles) and not immune but no growth (open circles) when exposed to natural mixed-species nematode infection

Immunoglobulin G

Mean optical density for *T. colubriformis* L3-specific IgG responses are given in Figure 3.17. Overall, immunoglobulin IgG responses showed a subclass by time interaction (P=0.028) reflecting a rise which was similar in magnitude in all subclasses but was earlier for groups that were immune regardless of growth.

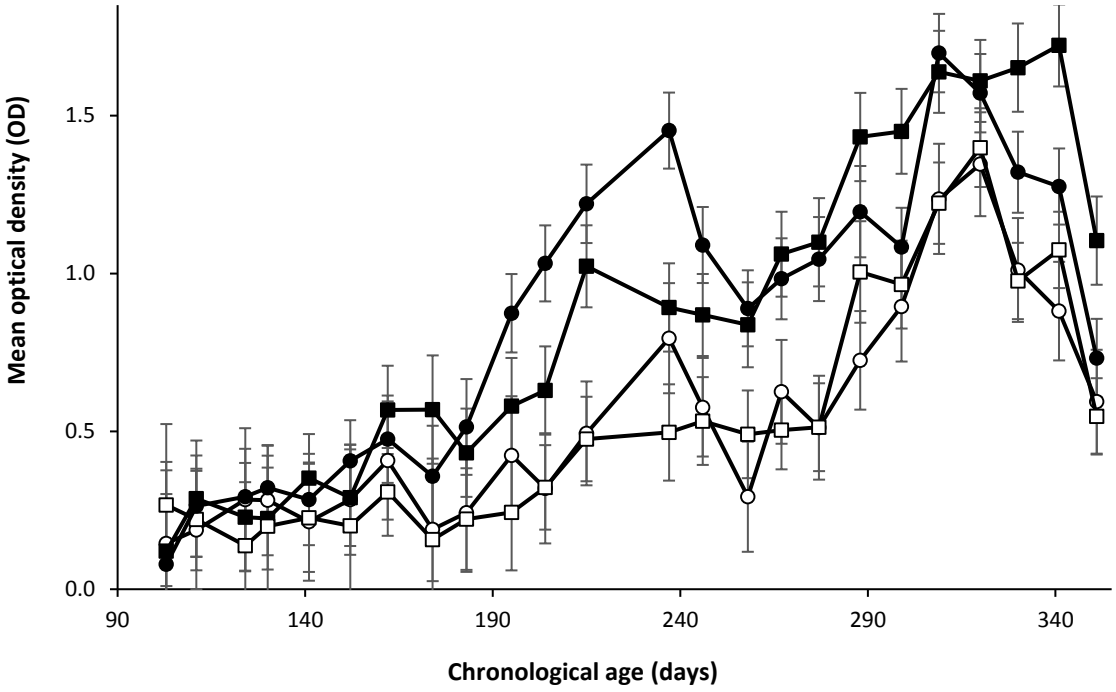


Figure 3:17 Mean optical density (OD) for *T. colubriformis* L3-specific IgG for Romney lambs re-grouped into four subclasses: immune and growth (closed squares), growth but not immune (open squares), immune but no growth (closed circles) and not immune plus no growth (open circles) when exposed to natural mixed-species nematode infection

Immunoglobulin A

Mean optical density *T. colubriformis* L3 IgA responses are given in Figure 3.18. Immunoglobulin A was greater in animals that were immune regardless of growth between days 195-250 compared with animals that were not immune regardless of growth (P=0.028) but eventually increased (P<0.001) in all groups to reach similar peaks at 300 day-of-age before declining.

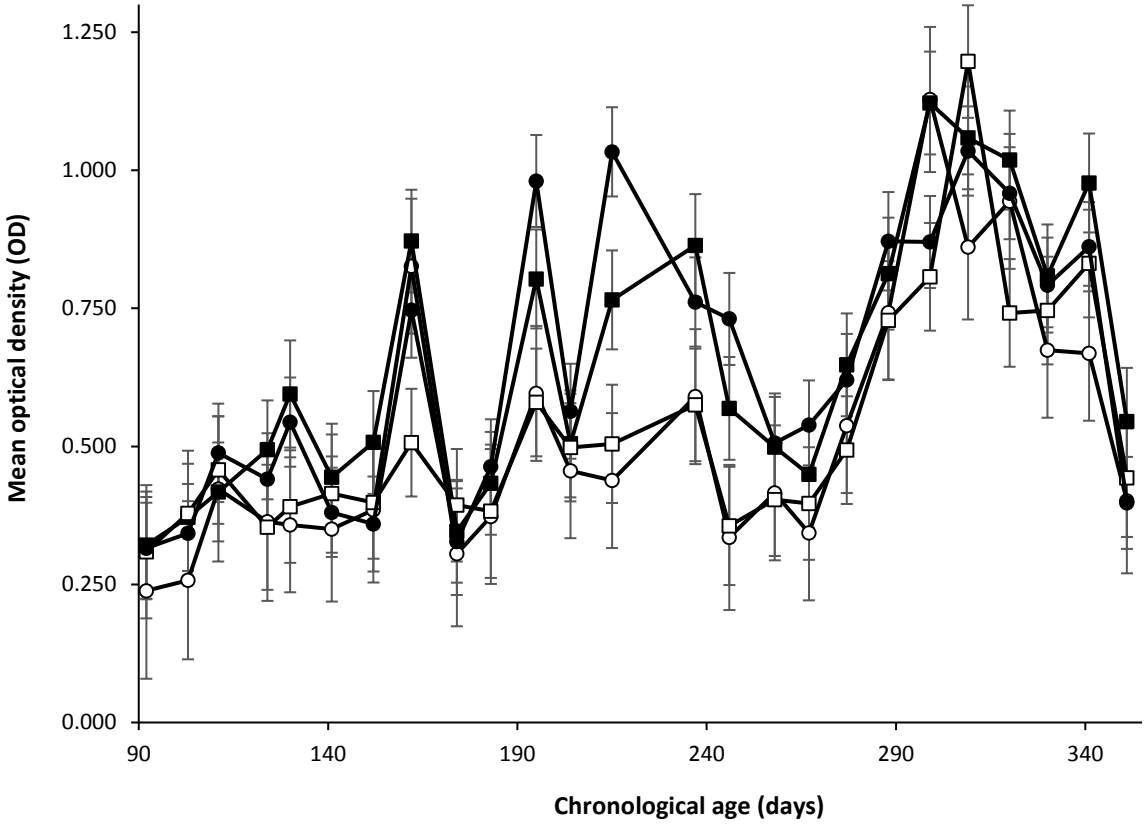


Figure 3:18 Mean optical density (OD) for *T. colubriformis* L3-specific IgA for Romney lambs re-grouped into four subclasses: immune and growth (closed squares), growth but not immune (open squares), immune but no growth (closed circles) and not immune plus no growth (open circles) when exposed to natural mixed-species nematode infection

Live weight (kg)

Mean fasted live weight (kg) is summarised in Figure 3.19. Overall, live weight showed a subclass by time interaction (P<0.001) which reflected a consistently greater but similar live weight in animals with growth regardless of immune status compared with the other two subclasses but being lowest in animals which were immune but with no growth. A clear demonstration of the lines was apparent based on weaning weights whereby 75% of animals in the high growth had a weaning weight above 23 kg.

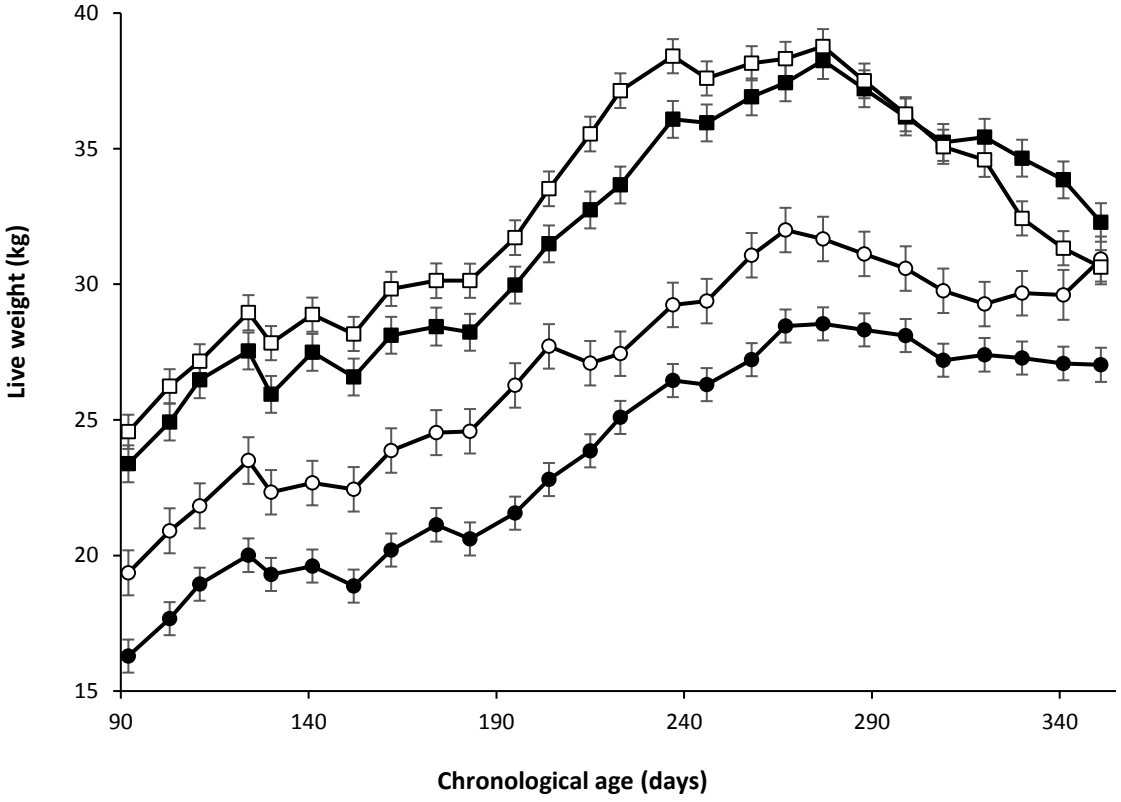


Figure 3:19 Mean fasted live weight (kg) for Romney lambs re-grouped into four subclasses: immune and growth (closed squares), growth but not immune (open squares), immune but no growth (closed circles) and not immune plus no growth (open circles) when exposed to natural mixed-species nematode infection

3.4 Discussion

It is apparent from the parameters measured here that there are commonalities but also differences between the selection lines. Both lines showed similarities in the overall response to GIN infection with both lines reaching similar magnitude of peak immunological responses. These results are in contrast with previous reports in animals from these lines which suggested that the magnitude of immune responses are typically greater in resistant compared with resilient lambs (Bisset et al., 1996b; Douch et al., 1995; Douch et al., 1994; Green et al., 1999; Morris et al., 2000; Shaw et al., 1999). It is a commonly held view that resilient line animals have a lesser immunological capacity when compared with their resistant counterparts, but this was not the case in the current study although the timing of immune development did differ. Further, both lines also showed similar overall pattern in both liveweight and liveweight gain and they had similar production levels and even FEC by the end of the study. Similarly, these observations were not consistent with the suggestion that resilient animals have greater performance than resistant animals when both are grazed together and exposed to similar parasite challenge (Bisset et al., 1996a; Bisset et al., 1996b; Morris et al., 2000; Morris et al., 1997). To this end, there was clear suggestion that despite decades of divergence selection for differences in response to parasites, the animals from both lines appeared to retain some fundamental characteristics suggestive of their common shared origin.

Differences did exist between the selection lines, however they appeared to be temporal. There was a clear and expected difference in the magnitude of the indicator trait of infection between lines, with mean back-transformed FEC increasing rapidly to reach a peak of >1500 epg by day 288 in resilient line animals whereas FEC for resistant counterparts typically remained relatively low <200 epg. Surprisingly, the corresponding immunological responses and particularly IgG remained low in resilient line animals up to day 260 while in resistant animals, IgG rose rapidly from day 200 and was greater suggesting that the low observed FEC in these animals may be reflecting immune responses that occur earlier than rises in circulating immunoglobulin. These observations are consistent with indoor studies with animals from these selection lines which showed a similar peak but a delayed rise in circulating parasite-specific IgA immunoglobulins in resilient animals (Greer et al., 2018) and provides further support that the animals selected for resistance have an earlier onset of immune development. Further, clear differences in animal performance were observed between the selection lines in the early phase, with resilient lambs exhibiting greater mean live weight and live weight gain compared with resistant lambs, although much of this reflected a greater weaning weight. Up to day 280, profiles in live weight in the current study were consistent with previous reports (Bisset et al., 1996a; Bisset et al., 1996b; Morris et al., 2000; Morris et al., 1997). The lower performance in resistant line animals may presumably have reflected the nutrient partitioning as proposed by Coop and Kyriazakis (1999) with resources prioritized to an effective immune response to parasitism that

guarantee the animal's survival in the short-term and a reduction in tissue deposition. Although, without measures of growth potential, this cannot be substantiated. Contrary, the performance of resilient lambs indicated that acceptable growth rates can be maintained during this phase despite a substantial rise in faecal egg count indicative of greater parasite loading. Acquisition of immune development is known to induce substantial nutritional penalty on the host animal (Greer et al., 2005) which may have negatively impacted on the productivity of resistant animals. The impact of the supposed nutritional stress was evidenced by lesser proportion of resistant-line animals (20%) that reached the slaughter weight of 35 kg at 215 day-of-age compared with 80% of resilient animals which reached this weight at the same age. The failure of animals to attain a given slaughter weight, suggests animals may have to stay longer on-farm which may affect both resource use efficiency and the overall profitability of the farming enterprise (Brown, 1990; McIvor & Aspin, 2001; Rendel et al., 2013; Thompson et al., 2016). Unfortunately, the protocol used in the current study did not factor in removal of animals at a given slaughter weight, and even if it did, the differences in the production advantage for resilience may be exemplified. From day 288 onwards, however, both resistant and resilient lambs had similar live weights which suggests either that resistant lambs were recouping benefits of the earlier nutritional investment of immune development (Greer, 2008) or that greater levels of parasitism were being felt and impacting on resilient animals, an effect which may have been exacerbated by the winter grazing regime which restricted nutrient supply and may have resulted in uncompensated pathological disturbances (Hoste et al., 2016). The greater decline in performance coincided with a rapid increase in immunoglobulin G in resilient lambs and a corresponding reduction in the FEC which suggested more nutrient resources may have been directed towards effecting a strong immune response to counter effects of parasitism at the expense of growth.

It is clear that although the magnitude of immune development in the parameters measured here was similar, the timing appeared to occur earlier in resistant than resilient animals. From the current study, resistant line animals were observed to have an earlier rise in immunological resoponses and cumulative percentage of animals that reached the threshold IgG of 1-OD but were associated with lighter weights relative to resilient counterparts. This suggests early onset of immune acquisition at a lighter weight and its associated nutritional costs. In addition, there was an earlier rise in the proportion of resistant female animals that reached their sexual maturity indicated by presence of mating marks. Further, relative maturity appeared to increase at a faster rate compared with resilient females between days 250 and 300, although the initial values were similar. Combined, this can be interpreted that at the same chronological age, animals selected for resistance are physiologically more advanced than their resilient counterparts. Given the temporal nature of immune development, both FEC and immunological measurements which occur at a set

chronological age, do not take into account physiological age. Physiological or relative maturity has been suggested to be associated with the development of immunity (Greer & Hamie, 2016; Taylor, 1965, 1968, 1980). This reflects correlations between the stage of animal growth, nutritional requirements, corresponding accumulation of fat deposition and the resulting adipocytokines (Ahima et al., 1997; Blache et al., 2000; Boisclair et al., 2000; Delavaud et al., 2000; Delavaud et al., 2002; Greer et al., 2009a; Lord et al., 1998). However, age and weight are confounded, and variations exists in the timing of maturity in body weight between breeds and even individual animals with similar weights (Taylor, 1980; Taylor & Fitzhugh, 1971) being earlier in lighter weight and longer in heavier animals. This suggestion implies that the often observed differences in the immune development may, in part, be attributable to the differences in animals physiological maturity rather than soley difference in magnitude of immune response.

There was a suggestion of sexual dimorphism between the selection lines in the response to parasite challenge. Although there was no interaction between selection line and sex, disparity in the live weight between males and females was evident in resilient, but not resistant lambs. Resilient males were heavier compared with their resilient female counterparts in the period preceding peak FEC, but both displayed similar low immunological IgG responses to infection. In contrast, males and female resistant lambs had similar liveweight, FEC, and immune responses throughout. Although Barger (1993) suggested that male sheep are more susceptible than females to both experimental and natural infections, this observation did not occur in the current study as both males and females within each line showed similar trends and magnitude in levels of infections. Sexual dimorphism is a commonly observed phenotypic difference between males and females in growth (Badyaev, 2002; Gatford et al., 1998; Lovich & Gibbons, 1992). However, it remains unclear why sex differences in growth, FEC and IgG did not occur in resistant-line animals. Further, the absence of clear definitive advantage in immunological responses in resilient females with lighter body weight compared with their resilient males may suggest that immunity is independent of body weight *per se* or that there are other factors (such as genetic) that have primary effects on immune development. The difference in body weight between male and female resilient lambs also appeared to be temporal with both sexes becoming similar from day 330 which may be suggestive that males were being impacted more by the combine effects of declined winter-feeding regime and parasitism compared with their female counterparts. These temporal sex differences may lead to different conclusions depending on the timing when such comparisons are made. An early assessment of body weights would favour males with greater rates of growth whereas a later (June onwards), may favour females as they appear to be less impacted by a combined effect of winter-feeding regime and costs of immunity than males, although on-farm by this time males may have been sent for slaughter.

Overall however, the current study appeared to suggest that selection for resilience in Romney sheep may have favoured growth in males and not in female lambs, but this phenomenon was not evident in resistant selection lines.

In summary, immune development occurred in both selection lines despite years of selection for divergence in the response to parasite challenge but the timing of which differed being early and more rapid in resistant line animals compared with a much-delayed response in their resilient counterparts. The differences in the timing of immune development appeared to be associated with physiological maturity which was more advanced in resistant than resilient animals. Furthermore, the nutritional costs associated with the acquisition of immunity appears to account for the temporal growth advantage in resilient lambs early in time as resistant animals would been expected to invest a substantial amount of their nutrition resources towards immunity, a phenomenon that was reversed when resilient animals developed immunity. The similarity in performance between lines in the later phase partly reflects a presumed greater nutritional stress and costs of immune development in resilient lambs and / or the increased growth of resistant line animals which suggested apparent benefits of their ability to recoup nutritional investments.

3.4.1 Implications

Differences in the timing of immune development during an infection in Romney sheep lines has implications on animal performance. Resistant line animals had lighter liveweight compared with resilient lambs starting from weaning up to day-330 and this difference in growth was consistent with the timing of immune development. These results contrasts computer prediction models that predicted 3.0 kg gain in live weight and 0.3 kg increase in fleece weight from selecting for resistance in Romney sheep (Leathwick et al., 1998) possibly because selection lines grazed together as opposed to separately, which was a necessary step of this study to ensure similar parasite challenge. If selection for resistance can only achieve reduced FEC without demonstrable corresponding increase in production traits (Bisset et al., 2001; Howse et al., 1992) they may not be attractive under commercial farming conditions whose aim is to increase farm productivity and profits (Brown, 1990; McIvor & Aspin, 2001; Rendel et al., 2013; Thompson et al., 2016). However, selection indexes as developed and supplied by SIL through WormFEC service, do provide an opportunity for both resistance and production traits to be concurrently selected to commercial ram breeders (McEwan, 1994; McEwan et al 1995).

Variations in immune development is worthy of exploiting in both selection programmes and commercial farms. On the one hand, the earlier but temporal greater growth potential in resilient lambs, despite harbouring moderately high parasite loads would be beneficial in the short-term to commercial farms with a goal to quickly finish lambs, although in the long-term, they may contribute

to greater pasture contamination. The desire for lambs with increased growth rates is targeting the high premium prices which typically occur earlier in the season (McIvor & Aspin, 2001).

The greater proportions (over 80%) of resilient males that reached a slaughter weight of 35 kg at about 200 days-of-age reflects the genetic merit for greater growth rate which was not apparent in resilient females and / or resistant counterparts although an early nutritional cost of immunity in resistant animals may have a contribution to these differences. However, the substantial reduction in productivity from the presumed greater effects of parasitism and associated nutritional stress on the already limiting nutrient supply of winter-feeding regime, may mean considerable additional measures would be required to maintain acceptable growth in absence of anthelmintics. This suggests that selection for resistance may be more appropriate for animals that are meant to be kept for longer period on the farm (such as replacement stock), to considerably reduce both pasture larval contamination for subsequent grazing and reliance on anthelmintic treatment for management of nematode infections. However, pasture contamination advantages of selection for resistance to nematode parasites could not be assessed as both selection lines grazed together. It appears imperative to incorporate both short and long-term production objectives to ensure greater economic returns from these Romney sheep lines. Finally, but not least in importance, is that selection for resistance has inadvertently favoured animals that are more immunologically mature, and possibly more physiologically mature at the same chronological age. Thus, until the timing of imposing selection pressure for nematode resistance is changed to allow both lines to reach the acquisition stage of immunity, animals with poorer growth potential may be perpetuated but these animals may not be favoured in commercial farming enterprises.

3.4.2 Opportunity for combined selection

Re-classification of these individuals demonstrated that there is variation in responses within lines that may provide opportunity for selection within Romney lines to incorporate growth in resistant-line animals and to include immunity within resilient selection-line animals. Greater differences exist in the growth between animals that were both immune and grew well compared with animals that were immune but did not grow well in each selection lines as shown in Figures 3.13 and 3.14 above for resistant and resilient selection lines, respectively. Interestingly, in both selection lines, animals that were both immune and grew well had weaning weights >23 kg compared with those that were immune but did not grow well whose mean weaning weights were below 20 kg despite both animals showing similar patterns and magnitude of immunoglobulin IgG responses.

While selection for nematode resistance trait alone appears to have favoured animals with poorer growth potential (Greer & Hamie, 2016) which is also apparent in this study, this has previously been attributed to the greater costs associated with acquisition of immunity (Bisset et al., 1996a; Douch et

al., 1995; Douch et al., 1994; Green et al., 1999; Greer et al., 2005; Shaw et al., 1999). However, the similarity in immunological IgG responses does suggest that other factors are involved. The results appear to suggest that opportunity exists to select for both enhanced immune response and growth traits as evidenced by existence of 30.4% and 26.7% of such animals in resistant and resilient Romney selection line that show similarities in patterns of growth and immunoglobulin G responses as provided in Figure 3.15. From this perspective, it would be of interest to observe the phenotypic expression of the F1 generations from crossing animals that are both immune and grow well from both selection lines although this was outside the scope of this study. These observations are consistent with previous report by Wheeler et al. (2008) who compared four Romney sheep lines namely; resistant, resilient, unselected control line and a line selected for an index combination of high growth and low FEC with all lambs grazing together and exposed to similar levels of nematode infections. The index line showed greater growth in both live weight and fleece weight of 3.5% and 6.8%, respectively, above what was hypothetically expected of animals from crosses between resistant X resilient in the absence of heterosis. In addition, FEC in Index line animals were reduced by 50% compared with both control and resilient animals but still higher than resistant. Together, these findings provide further support for the suggestion that combined selection for both resistance and growth is possible (Bishop & Morris, 2007; Bisset & Morris, 1996) but the resulting genetic relationship depends on the balance between the two seemingly opposing traits (Bishop, 2012; Bishop & Stear, 2003) as both traits (immunity and growth) compete for nutrient resources in their expression (Coop et al., 1979; Coop & Kyriazakis, 2001). While it appears applicable to strike a balance between immunity and growth under conditions where species such as *T. colubriformis, T. circumcincta* are most prevalent, the scenario may be different in environments where the highly pathogenic species *H. contortus* is dominant as the opportunity costs of not developing immunity can be expected to be greater (Riley & Van Wyk, 2009; Van Wyk & Bath, 2002).

Animals that are not immune and or those that do not grow well in both selection lines are cause for concern. Nearly 70% of animals in resistant selection line that did not grow well raises more concerns of a continued selection of nematode resistance based only on indicator traits. Similarly, the presence of 72.4% of animals that grew well but were not immune in resilient-line animals will gradually push the phenotypic response toward susceptibility and animals that may require routine anthelmintic treatment to maintain acceptable productivity under nematode parasite infections. As too strong an immune response evokes unwanted immunopathology and associated nutritional penalty on the host animal (Graham et al., 2005; Greer et al., 2005; Williams, 2011), selection for increased productivity has often been observed to increased susceptibility of animals to nematode infection (Eady et al., 1998; McEwan et al., 1997; Morris et al., 2010).

These results point to the urgent need for enforcing implementation of combined selection tools in breeding and commercial farms to identifying animals with both greater growth potential and moderate levels of immunity to nematode infections. Bearing in mind that producers will not be satisfied with animals that only express enhanced resistance to parasites but with no demonstrable associated benefits in productivity (Bisset et al., 2001; Howse et al., 1992). Interestingly, it appears that weaning weight could be used as a predictor for animals that are likely to grow well.

3.4.3 How to identify animals with both resistance and greater growth potential

The ability to identify animals that maintained good productivity and low FEC is considered highly desirable for increasing production potential of the sheep industry and for the sustainable management of nematode parasites. Identifying such animals, would require use of weaning weights at set threshold of >23 kg followed by sampling for either immunoglobulin G at about 220 days-of age or FEC at about 270 days-of-age as shown in Table 3.3. Twenty-six percent (26.4%) of the 53 Romney lambs combined had such attributes with males and females equally represented. These animals had an early rise in both IgG and IgA responses however, their growth profiles were similar to those animals that grew well but were not immune suggesting the cost of immunity and subsequent diversion of nutrients may not be the sole cause of reduced performance in animals that are selected for resistance. There was a considerable difference in mean weaning weights (7.1 kg) between animals with immunity and growth versus those which were immune but did not grow well, which suggests that animals that are likely to grow well, can be determined at weaning. The other advantage of animals that are immune and grow well (>23 kg) is that they maintained similar low FEC which is a desirable characteristic in an effort to reduce pasture contamination with infective larvae which contrasted with the substantially greater FEC from animals that grew well but were not immune. Although there was a temporal difference in rate of increase in growth profiles between days 200 and 240 relative to those with growth but not immune, which was suggestive of costs of a greater and early immune activation in animals that grew well and were immune, a difference in the rate of decline in weight later in the growth phase was less than their counterparts. Interestingly, there was no apparent indication from animals that were immune but with below average growth that their late phase growth rates were in the process of recouping the benefits of earlier nutrient investments in immunity. This clearly suggests that animals with less than 20 kg are likely not to grow well but are expected to exhibit greater immunological capacity at least in Romney sheep. Overall, these results have demonstrated clear potential for combined selection aimed at achieving both increased growth and enhanced immune response to nematode parasites, the product of which should be acceptable to commercial producers and lead to improved productivity of the sheep industry and sustainable control of the impacts of parasitism.

Table 3:3 Possibility of combined modelling for greater growth potential and immunity to nematode parasite infection using weaning weights (kg), immunoglobulin G at 220 days and sampling for FEC at 270 day-of-age in Romney sheep lambs grouped in four subclasses: 1) immune and growth (+/+), 2) not immune and growth (-/+), 3) immune with no growth (+/-) and 4) not immune and no growth (-/-)

Weaning weights	Sex	Subclass	# of animals	# of animals with FEC >500 at day 277	Percentage (%) within sex group	Mean LWG (kg) at day 277	Mean FEC (epg) at day 277	Mean LWG (kg) at day 351	Mean FEC (epg) at day 351	Mean age at 1-OD IgG
> 23 kg	Female	+/+	4	1	25	12.2 ± 1.2	200 ± 168	6.7 ± 0.6	221 ±65	241
		+/-	0	0	N/A	N/A	N/A	N/A	N/A	N/A
		-/+	3	2	67	11.1 ± 2.2	1300 ± 874	5.5 ± 1.3	570 ± 265	288
		-/-	0	0	N/A	N/A	N/A	N/A	N/A	N/A
		Sub-total	***7***							
	Male	+/+	6	2	33	16.7 ± 0.9	300 ± 179	9.7 ± 0.9	265 ± 55	214
		+/-	0	0	N/A	N/A	N/A	N/A	N/A	N/A
		-/+	12	10	83.3	14.4 ± 0.5	2483.3 ± 598.7	8.4 ± 1.0	957.2 ± 131.7	288
		-/-	1	1	100	12	3700	5.4	968	288
		Sub-total	***19***							

Chapter 4

Does the epidemiological benefit from resistant animals out-weight the costs?

4.1 Introduction

Selection of animals that are genetically either resistant or resilient to gastro-intestinal nematodes (GIN) are two divergent approaches, the benefits of either are a debated option for sustainable parasite control. Resistant animals will excrete fewer eggs on pasture likely leading to an epidemiological benefit (Bisset et al., 1991c; Bisset et al., 1997b; Li et al., 2001; Miller et al., 1998; Morris et al., 2000; Morris et al., 1997). Predicting the actual benefits of selection and or breeding for resistance is not always clear due to complex interactions between parasite epidemiology, management practices and host resistance (Bishop & Stear, 1997). Mathematical simulation models have predicted substantial reductions in faecal egg count (FEC) by approximately 40% and pasture contamination by ~83% by grazing resistant animals separately, while grazing susceptible (high FEC) animals separately would increase pasture contamination by ~240% (Laurenson et al., 2012), suggesting potential indirect benefits on animal performance from resistance would be expected. Similarly, Leathwick et al. (1998) predicted 3.0 kg gain in live-weight and 0.3 kg increase in fleece weight at 12 months of age from selecting for resistance if faecal egg count and subsequent contamination was to be reduced by 50%. However, a majority of the assumed consequences of greater contamination are based on the presumption that production loss is directly proportional to larval challenge (Coop et al., 1982; Dobson et al., 1990c; Symons et al., 1981) which may not be the case in these selection lines as the threshold of which they develop immunity and its associated costs may differ.

In New Zealand, selection solely for resistance in dual-purpose breeds has been associated with poorer animal production in terms of live weight and liveweight gain compared with selection for resilience (Bisset et al., 2001; de Lautour & de Lautour, 2010; Wheeler et al., 2008). Although resistance may confer an epidemiological advantage, it is not clear whether this outweighs the reported poorer growth potential often observed when resistant animals are grazed together with resilient or susceptible counterparts. There is evidence that physiological differences may exist between the lines, particularly, as study 1 (Chapter 3) showed in the timing of immune development. Given the cost of parasite infection in ruminant animals has been attributed to the acquisition phase (Greer et al., 2008; Greer, 2008; Greer et al., 2009c), this may be considerable. Previous studies investigating the production differences between lines of animals selected for resistance or resilience

have reported greater levels of productivity in resilient animals (de Lautour & de Lautour, 2010; Morris et al., 2000; Morris et al., 1997; Watson et al., 1992; Woolaston, 1992). These comparisons have often been made where lambs of each phenotype are grazed together and may have resulted in underestimation of the impact of host genotype on LW, LWG, FEC, and pasture contamination due to potential masking effects. Initial studies by (Bisset et al., 1997a) compared nematode population dynamics and lamb productivity in Romney lambs selectively bred for resistance or susceptibility to parasite infection in rotational grazing farmlets where all lambs (except selected groups) received a minimum periodical anthelmintic drench. The authors reported that the potential epidemiological benefits of resistance were not associated with large advantages in animal performance. In the present study, the epidemiological and animal performance benefit of selecting for resilience or resistance to GIN parasites was evaluated using Romney sheep from established selection lines grazed either separately and or mixed in farmlets.

4.2 Materials and methods

The study was carried out at the Lincoln University Ashley Dene farm with approval and in accordance with the LUAEC #637. Ten 0.3ha fully irrigated plots with newly established pasture were allocated to one of four farmlet treatments providing low initial levels of pasture larval contamination with each farmlet consisting of either three or two paddocks. Prior to lactation in September 2015 and 2016, the farmlets were stocked with 8 and 6 pregnant ewes, respectively, from the resistant and resilient Romney lines. Lambs were born in September 2015 and 2016, and weaned at 10 weeks of age at which point the ewes were removed and the lambs remained set stocked in their respective birth paddocks until 210 days post birth. Each paddock represented a replicate in farmlet treatment based on either selection line or anthelmintic regimes, as described below. Lambs were removed from the paddock early April of each year and the paddocks rested until required in August for the next lambing.

4.2.1 Farmlets

Treatment1: Resistant Romney line without anthelmintic treatment (RtA). The objectives of this treatment group were to evaluate the performance and epidemiological characteristics such as population dynamics of a farmlet solely consisting of resistant animals without anthelmintic intervention.

Treatment 2: Resilient Romney line without anthelmintic treatment (RlA). The objectives of this treatment group was to evaluate the performance and epidemiological characteristics of a farmlet solely consisting of resilient animals without anthelmintic intervention.

Treatment 3: Resilient and resistant Romney lines grazed together with anthelmintic treatment (Mxd+Dr). At paddock level Mxd+Dr represented two groups based on selection line as Rl+Mxd+Dr and Rt+Mxd+Dr respectively. Ewes were set stocked prior to lambing in two paddocks during lactation, post-weaning lambs remained in the paddock and ewes were removed. Each paddock was stocked with 4 resistant and 4 resilient ewes in 2015 and 3 resistant and 3 resilient ewes and their new born lambs in 2016. Lambs were given long-acting Cydectin injection anthelmintic drench twice, first at weaning in Novermer and 3-months later at the end of Mach. The objective of this farmlet was to provide a comparison of the potential growth rate able to be achieved when parasites are not present (comparison of Rt and Rl with paddocks Rt+Mxd and Rl+Mxd treatment groups).

Treatment 4: Resilient and resistant Romney lines grazed together without anthelmintic treatment (Mxd). At paddock level (Mxd) representing two groups based on selection line as Rl+Mxd and Rt+Mxd respectively Ewes set stocked in two paddocks prior to lactation, post-weaning lambs remained in the paddock and ewes were removed.Each of the two paddocks paddock was stocked

with 4 resistant and 4 resilient ewes without anthelmintic treatment at each year. The objective of this farmlet was to provide a growth rate comparison of the lambs to previous research that has been carried out when animals of these selection lines were run together. This allowed for a comparison of the over or under estimation of the benefits of selection for resistance (when comparing with treatments RtA and RlA compared with paddocks without anthelmintic use).

4.2.2 Animal sampling

Sampling of lambs in each farmlet occurred from weaning at mean age of 90 days and then every three weeks until lambs were 210 days-of-age. At each time, faecal samples for the determination of the concentration of nematode eggs in the faeces, live weight, body condition score and saliva samples for the determination of parasite-specific antibody levels were collected. Faecal samples were collected from the rectum of each lamb immediately upon yarding for the determination of the concentration of nematode eggs in the faeces, and differentiation between Strongyle and Nematodirus species using a modification of the McMaster method, with a sensitivity of 100 eggs per gram (epg). Dag scores (breech soiling) was determined using a dag score chart (Beef & Lamb SIL 2017) with using a 6-point scoring scale ranging from 0 (no dags) to 5 (very daggy) as summarized in Figure 4.1. Saliva samples were taken using mouth swabs that were then centrifuged at 1200 x g and the saliva stored at -20 ^{o}C until analysis. Animal live weights (LW) were recorded with the use of electronic identification tags (Allflex New Zealand) and a Edit Display Wand tag reader connected to a Tru-test XR3000 (Tru Test Ltd, NZ) head unit. After sampling, animals were immediately returned to their respective grazing paddocks.

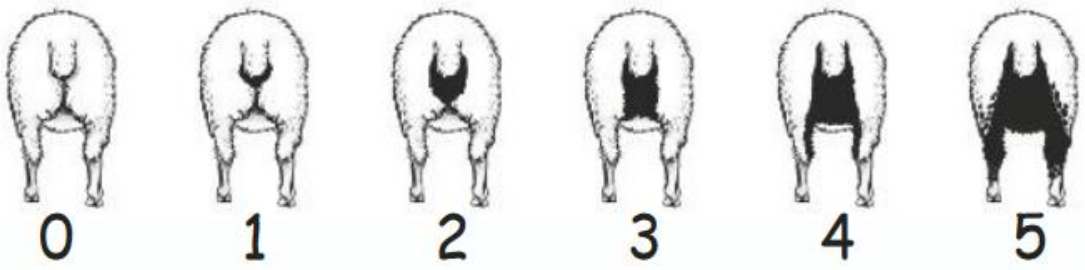


Figure 4:1 Dag score chart: from Beef & Lambs NZ, Dag score SIL Technical Note, 2017.

4.2.3 Pasture Sampling and processing

Herbage samples were taken for the determination of the concentration of infective larvae on pasture. Samples were collected by plucking pasture (close to ground level) between the thumb and index finger at every 3-5 steps while walking in zigzag transects across a paddock.

A minimum of 200 pasture clips from a single paddock constituted one composite sample for each paddock as described by (Leathwick et al., 2006). Samples were immediately weighed and then stored at 5^{o}C prior to processing. The bags containing grass were washed in a washing machine (Easy Pressure, Banmix Food Machine LTD, Auckland NZ) with four litres of lukewarm water for 200 revolutions, the passed through a coarse mesh sieve (aperture size 2 mm) into a 5 litre beaker. The grass was then dried in an oven at 70^{o}C for 72 hours with dry weights recorded. Following settling overnight the resultant suspension was siphoned and discarded. The sediments and larvae were then transferred to a measuring cylinder for a second sedimentation as described by Waghorn et al. (2002). Following siphoning, the volume of sediments was recorded and the content agitated until mixed. The larval suspension was then poured onto a 150 mm diameter filter paper (No. 2 Advantec, Tokyo, Japan) and allowed to dry until all the surface water has disappeared. The filter paper was thereafter inverted and placed on a baermann filter funnel. After a minimum of 36 hours, 100 ml of fluid was withdrawn and stored in glass bottles and stored at 4^{o}C. Cleaned samples were concentrated to 20 ml by siphoning and larvae present in two 1 ml samples were counted, differentiated into strongyle and Nematodirus species using a methods similar to those descibed by Van Wyk et al. (2004) and McMurtry et al. (2000) and results expressed as number of larvae per kilogram of herbage dry matter. Briefly, the shape of the "head" (cranial extremity) of the larva and the length of the sheath "tail" (the extension of the sheath from the tip of the larval caudal extremity to the tip of the tail of the sheath) were the distinguishing features used to identify the species. The cranial extremity (head) of *T. circuncincta* L3-larvae is smooth and flattened and has a slight "shoulder" close to its cranial tip while the head of *T. colubriformis* is tapered and shaped like a pencil but do not poses "shoulder". Contrary, the head of *Nematodirus* spp. is bullet shaped and flattened at the the tip and that of *Cooperia* has two refractile bodies in the head. Further, the lenth of whole body for *T. colubriformis* L3-larvae is shorter (about 30 µm) relative to that of *T. circuncincta* which is about 35 µm long whereas whole length of *Nematodirus* spp is on average 270 µm long. The length of shealth tail of *T. colubrifomis* is short and has 2-3 tubercles structures, that of *T. circumcincta* is slightly longer than *T. colubriformis* but lacks tubernacle whereas for *Nematodirus* the tail sheath is long relative to the other species. Although *Nematodirus* was not characterised into distinct species, *Nematodirus battus* does not exist in New Zealand (Brusdon, 1970; Vlassoff, 1973, 1976: Vlassoff et al 2001).

Herbage mass in kilograms dry matter per hectare (kgDM/ha) was estimated using a Rising Plate Meter (RPM) every three weeks from weaning until 220 days in the first year whereas in the second season, herbage was estimated three times only due to lack of herbage availability which reduced the accuracy of the rising plate meter due to presence of surface stones, two during lambing (one August and the other in September) and one 130 days post-weaning.

At each sampling, herbage height from 30 random spots in each paddock were measured by RPM and herbage mass estimated using the formula [mean RPM height (cm) X 200].

Pasture sub-samples obtained from each of the 10 paddocks in September of each year were freeze dried, ground to 1 mm particle size and scanned through the near infrared reflectance (NIR) spectroscopy for the determination of estimated nutritional composition of the herbage. One gram of composite forage sample from each of the 10 paddocks (and for each season) was exposed to an electro-magnetic scan (Foss NIRSystems) over a spectrum wavelength of between 1100 and 2500 nm. The energy absorbance identifies chemical bonds within the forage sample such as CH, -OH, -NH, SH etc, which then form the basis for identification of sugars, structural fibres, proteins, lipids and others (Ulyatt et al 1995 in Corson et al 1995).

4.2.4 ELISA analysis

Saliva samples were analysed for antibody IgG and IgA responses to L3-specific antigen for *T. colubriformis* and *Nematodirus* spp using an enzyme linked immunosorbent assay (ELISA) as described in Chapter 3 (Section 3.2.6). For both *T. colubriformis* and *Nematodirus Spp*., 50 µl of L3 and L5 antigen/well at 2 µg/ml in coating buffer (stock=300 µg/ml = >1:150 dilution) were incubated at 4^{o}C overnight in 96 well plates. Following washing 5 times with dilution buffer containing 0.1% (w/v) Tween 20 (W-T20), 200 µl/well blocking buffer (10 mM-phosphate buffer at pH 7.2 containing 0.5% Tween-20 and 5% bovine skim milk powder) was added to plates and incubated for 2 h at room temperature. Plates were then washed 5 times with washing buffer. Diluted saliva (1:10 for IgA and 1:100 for IgG) was added to ELISA plates at 50 µl /well, incubated for 2 h and then plates were washed 5 times with washing buffer solution. Rabbit anti-sheep IgG and or IgA conjugated with horseradish peroxidase (Bethly Laboratories Inc., USA), diluted 1:2000 with ELISA buffer, was added to each well (100 µl) and incubated for 1 h at room temperature. Plates were washed 5 times with washing buffer solution. To develop colour, 100 µl/well of tetramethyl benzidine (TMB) substrate was added and incubated for 40 minutes at room temperature. The substrate 0.05 M phosphate-citrate buffer pH 5.0 was made of 25.7 ml 0.2 M Na_2HPO_4 + 24.3 ml 0.1 M citrate and made up to 100 ml with deionised distilled water (dH_2O) to which 2 µl of 30% H_2O_2 + 1 TMB tablet per 10 ml buffer was added. The reaction was stopped by adding 100 µl/well of stop solution (6.9ml of 1.25M concentrated H_2SO_4) and then plates read for optical density at 450 nm using ELISA plate reader.

4.2.5 Statistical analysis

Live weight (LW) and live weight gain (LWG), faecal egg counts (epg), pasture larvae and immunological antibody responses and dag scores were subjected to sequential comparison for antedependence structures prior to analysis as repeated measures using a Restricted Maximum Likelihood (REML) model with grazing treatment groups (separate and un-drenched, mixed-grazed and drenched and mixed-grazed and un-drenched) selection line and time included as factors and differences determined using a least significant difference (LSD). Prior to analysis, LW values were adjusted for sex by adding the difference in mean LW between males and females at each sampling time to the female lamb weight. For cumulative liveweight gain (LWG) and weaning weights (WW), a general analysis of variance (ANOVA) was used. Data for FEC and pasture larval infestation were first log transformed (log10 (count+100)) before analysis and results reported as back-transformed means. Data on herbage dry matter (kgDM/ha) and NIR estimated nutrient composition of the pasture were analysed by general analysis of variation (ANOVA). Mean liveweight gain (LWG) for Rt+Mxd+Dr was used as benchmarks for calculating production losses from RtA and Rt+Mxd groups whereas mean LWG for Rl+Mxd+Dr was used to calculate loses RlA and Rl+Mxd groups respectively. For example, production loses of RtA were calculated as follows: [(Mean LWG of Rt+Mxd+Dr – Mean LWG of RtA) X 100)] / Mean LWG for Rt+Mxd+Dr.

4.3 Results

For 2015-2016 season, there was late establishment of pasture in the experimental plots due to initial failure of sown pasture to germinate. This meant that the pregnant Ewes were lambed elsewhere (between mid-August to September) and only lambs were introduced to the farmlets at weaning on November 20th, 2015 with a pre-existing infection. In the second season (2016-2017), FEC for one lamb (2090) in the Mxd+Dr treatment group remained high after first drench and had to be re-drenched later, so it was excluded in analysis. Parameters such as herbage mass and pasture larvae were measured at paddock level whereas FEC, LW, LWG, IgG and IgA were performed at individual animal level.

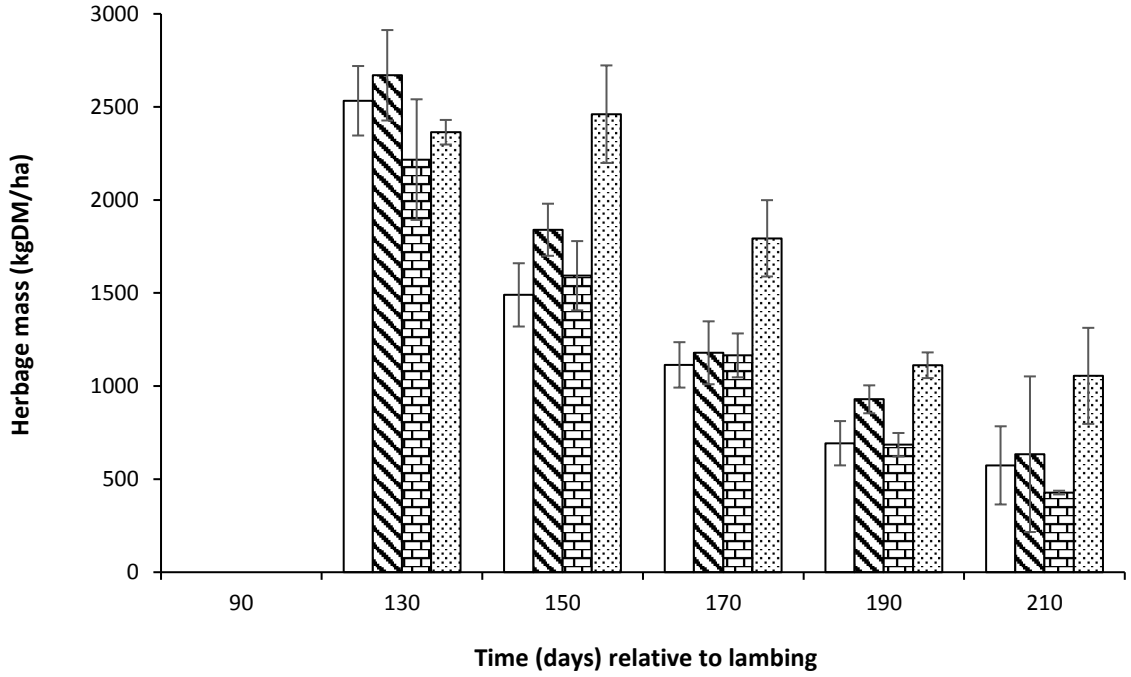
4.3.1 Pasture mass and quality

Herbage mass (kgDM/Ha)

Mean herbage mass yield (kgDM/ha) for the different farmlets are given in Figures 4.2a and 4.2b for 2015-2016 (season 1) and 2016-2017 (season 2), respectively. For 2015-2016 season, herbage mass during the entire study period declined with time ($P<0.001$) and was greater in RtA group (1757.3 ± 80.4 kgDM/ha) compared with RlA, Mxd+Dr, and Mxd groups (1217.8, 1280.9 and 1450.0 kg/ha) respectively ($P<0.001$).

During lambing time and a month preceding lambing in season 2, RtA and RlA groups had higher herbage mass (2138.4 ± 123.2 and 1777.9 ± 123.2 kgDM/ha) compared with Mxd+Dr (1245.5 ± 150.9 kgDM/ha) and Mxd treatment group (1312.5 ± 150.9 kgDM/ha) respectively ($P=0.002$). When sampled again on January 9, 2017 when lambs were 130 days old, there was no difference in herbage mass between the farmlets ($P=0.454$) with means of 1246.7, 1546.0, 1092.0 and 1350.0 kgDM/ha for RtA, RlA, Mxd+Dr and Mxd farmlets, respectively.

(a)



(b)

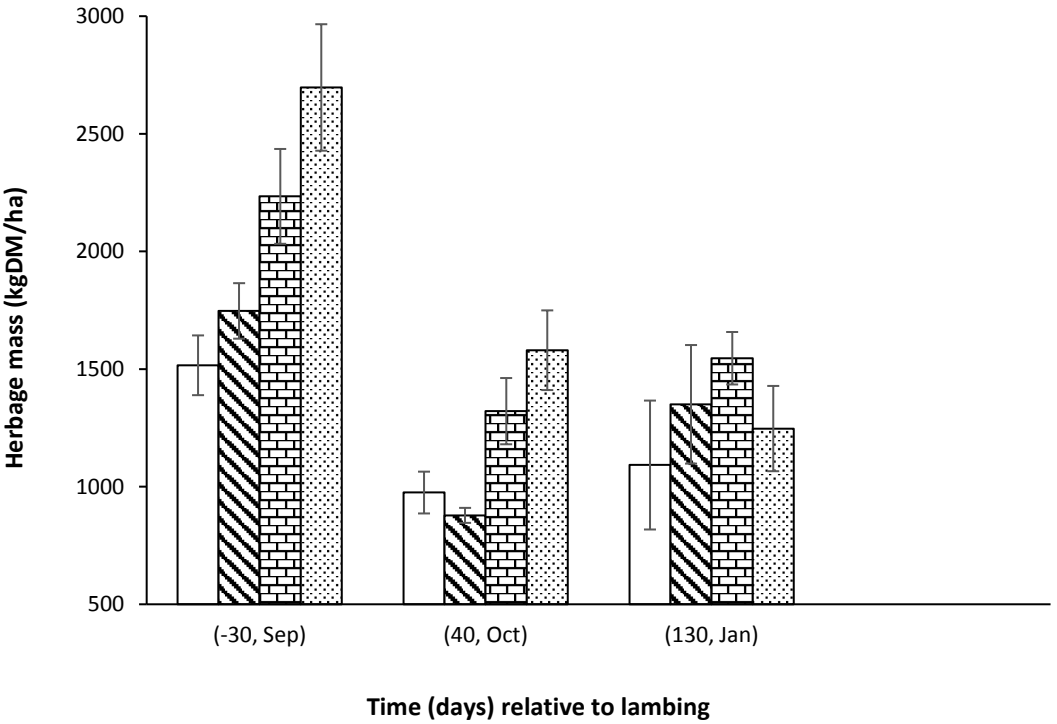


Figure 4:2 Mean herbage mass (kgDM/ha) for season 1 2015-2016 (a) and season 2 2016-2017 (b) in farmlets grazed by Romney lambs under natural mixed-species parasite infections: RlA (brick columns), RtA (dotted columns), Mxd+Dr (open columns) and Mxd (diagonal line columns)

Nutritional composition of herbage

Mean NIR estimated nutritional composition of herbage in farmlets grazed by resistant lambs alone, resilient lambs alone, mixed drenched lambs and mixed un-drenched lambs are given in Tables 4.1 and 4.2 for 2015-2016 and 2016-2017 seasons, respectively. The nutritional quality of pasture at the beginning of the study in the different farmlets were similar for most of the components and only protein (%) tended to differ (P=0.08) being greater in Mxd+Dr (10.5%) compared with a mean protein content of 8.7% for the other treatment groups respectively.

For season 2, the quality of the pastures at start of the study was similar except for organic matter % (P=0.055) and protein content (P=0.086). Percentage organic matter was higher in RlA (92.2%) and RtA (91.9%) whereas protein content was higher in Mxd (15.4%) and Mxd+Dr (14.9 %) groups compared with RtA (13.2%) and RlA group (13.6%), respectively.

Table 4:1 Mean NIR estimated nutritional composition of herbage in farmlets in 2015-2016 season: acid detergent fibre (ADF), carbohydrate (CHO), dry matter digestibility (DMD), dry organic matter digestibility (DOMD), neutral detergent fibre (NDF), organic matter (OM), organic matter digestibility (OMD), protein, rumen dry matter (rDM) and metabolizable energy (ME)

Components	RtA	RlA	Mxd+Dr	Mxd	SEM	P-values
ADF DM%	30.2	30.4	29.3	31.0	1.14	0.59
CHO DM%	25.4	25.4	23.6	24.6	1.28	0.45
DMD%	65.5	65.6	67.6	64.4	1.99	0.67
DOMD%	65.4	65.4	66.9	64.5	1.51	0.76
NDF DM%	52.7	52.8	51.2	53.1	2.21	0.29
OM%	93.3	93.5	92.6	93.9	0.81	0.59
OMD%	69.2	69.3	71.4	67.8	2.22	0.70
Protein DM%	8.8	8.8	10.0	8.5	1.09	0.08
rDM%	94.3	94.0	93.8	94.1	0.21	0.80
ME (MJ ME/kg DM)	10.5	10.5	10.7	10.3	0.24	0.76

Table 4:2 Mean NIR estimated nutritional composition of herbage in farmlets in 2016-2017 season: acid detergent fibre (ADF), carbohydrate (CHO), dry matter digestibility (DMD), dry organic matter digestibility (DOMD), neutral detergent fibre (NDF), organic matter (OM, organic matter digestibility (OMD), protein, rumen dry matter (rDM) and metabolizable energy (ME)

Components	RtA	RlA	Mxd+Dr	Mxd	SEM	P-value
ADF DM%	21.7	21.3	20.4	21.3	0.37	0.34
CHO DM%	29.5	29.3	28.0	26.9	1.24	0.12
DMD%	79.1	81.3	80.8	80.0	0.70	0.19
DOMD%	78.2	80.8	79.6	78.9	0.73	0.12
NDF DM%	40.6	41.0	37.6	39.4	1.21	0.30
OM%	91.9	92.2	91.0	91.4	0.31	0.06
OMD%	85.4	87.9	87.3	86.3	0.79	0.22
PROTEIN DM%	13.2	13.6	14.9	15.4	1.00	0.09
rDM%	94.0	94.1	94.1	93.9	0.11	0.65
ME (MJ ME/kg DM)	12.5	12.9	12.7	12.6	0.12	0.12

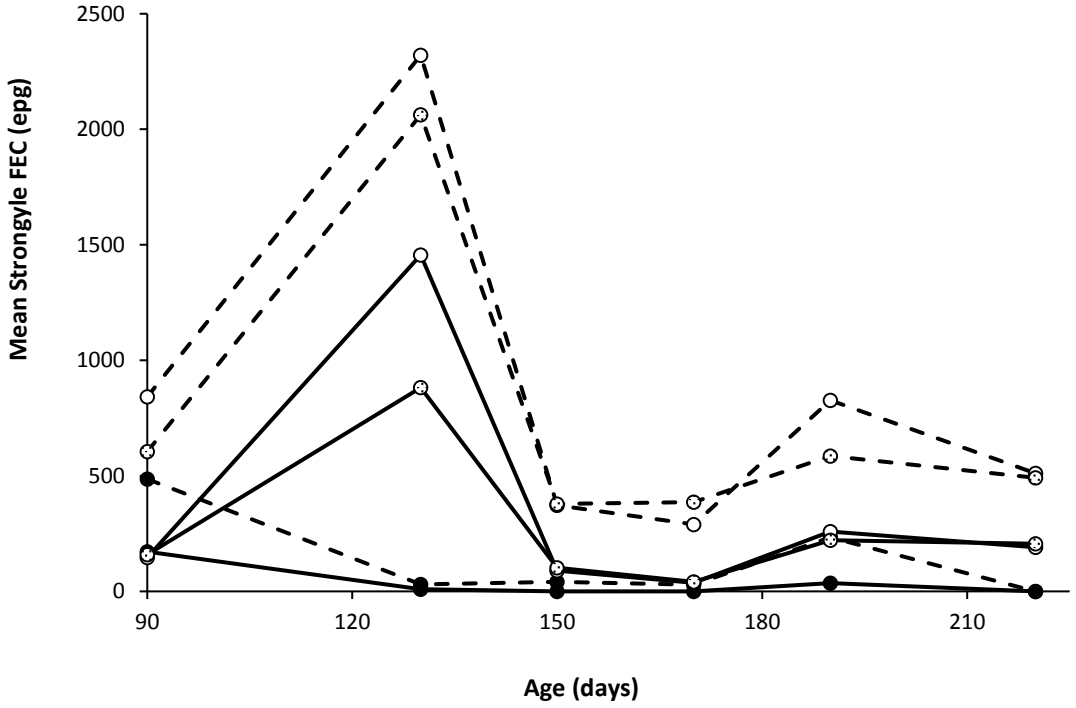
4.3.2 Faecal egg count (FEC; epg)

Strongyle FEC

Mean back-transformed *Strongyle* FEC for 2015-2016 (season 1) and 2016-2017 (season 2) are given in Figures 4.3a and 4.3b, respectively. In season 1, *Strongyle* FEC showed an interaction between grazing treatment and time ($P<0.001$) reflecting a rise in faecal egg count which peaked at 130 days-of-age in the RlA and Rl+Mxd whereas the RtA, Rt+Mxd, Rt+Mxd+Dr and Rl+Mxd+Dr groups maintained low FEC throughout.

For season 2, results tended to show an interaction between treatment and time ($P=0.058$) reflecting greater FEC in RlA and Rl+Mxd groups which also increased with time to reach peak of 800 and 500 epg respectively whereas FEC for RtA, Rt+Mxd and Rt+Mxd+Dr groups were consistently low.

(a)



(b)

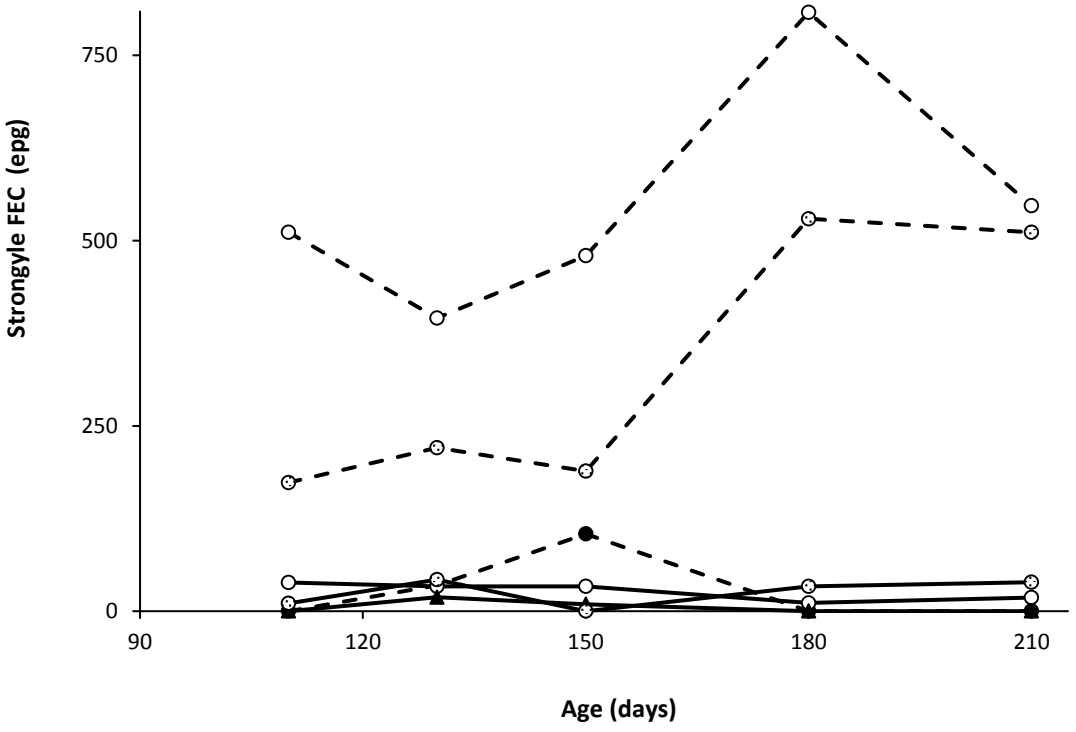


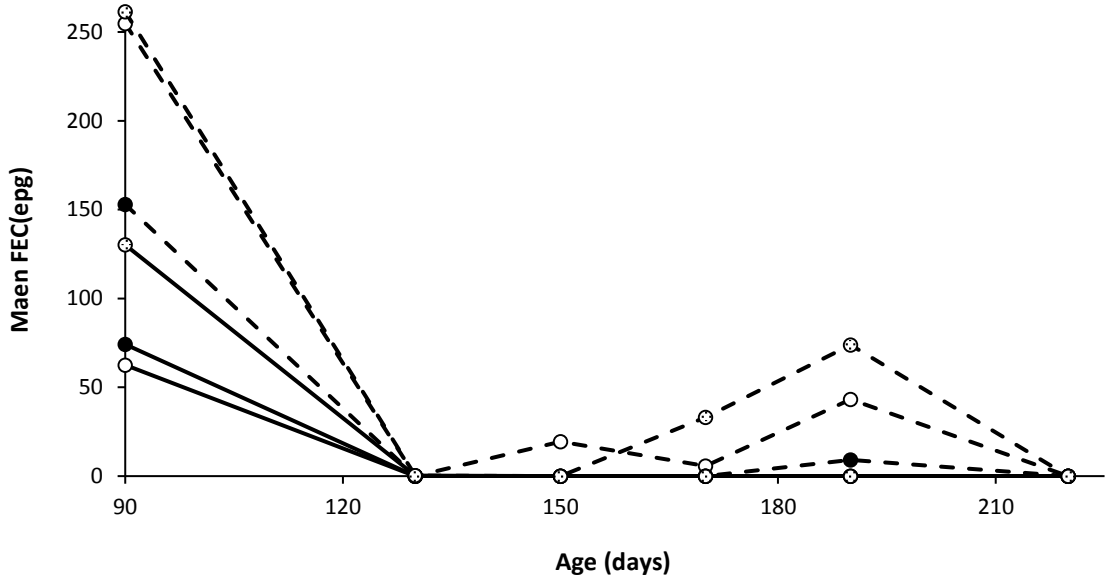
Figure 4:3 Mean back-transformed *Strongyle* FEC (epg) for (a) season 1 and (b) season 2 in Romney lambs under natural mixed-species parasite infections: RlA (dashed line, open circles), Rl+Mxd+Dr (dashed line, closed circles), Rl+Mxd (dashed line, dotted circles), RtA (solid line, open circles), Rt+Mxd+Dr (solid line closed circles) and Rt+Mxd (solid line, dotted circles)

Nematodirus FEC

Mean back-transformed *Nematodirus* FEC are presented in Figure 4.4a and 4.4b for 2015-2016 and 2016-2017 seasons, respectively. There were interactions between treatment and time (P<0.001) which reflected higher initial *Nematodirus* worm eggs which decline with time but there was no effect of treatment (P=0.427) despite infection being generally low.

For season 2, there was an interaction between selection line and time (P<0.001) reflecting higher initial FEC in lambs from RlA treatment group which decline with time while the RtA counterparts maintained lower FEC respectively. There was no interaction between selection line and treatment (P=0.147) and the interaction between treatment and time showed a tendency (P=0.090) reflecting a decline in FEC with time in RlA and Rl+Mxdr+Dr lambs whereas in Rl+Mxd group FEC rose on day 180, respectively.

(a)



(b)

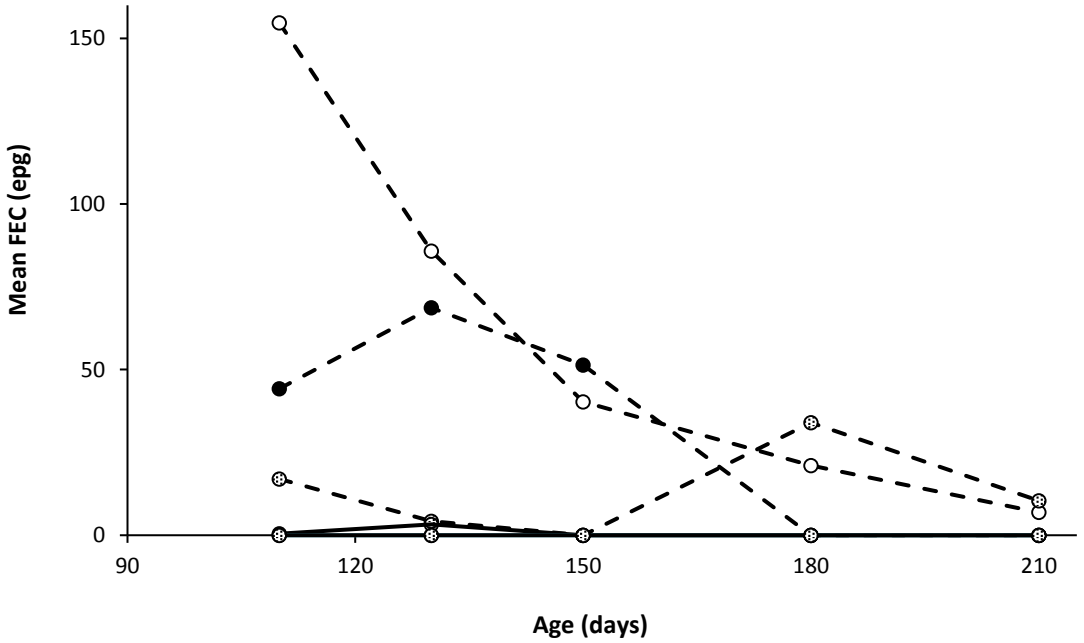


Figure 4:4 Mean back-transformed *Nematodirus* Spp. FEC (epg) for (a) season 1 and (b) season 2 in Romney lambs under natural mixed-species parasite infections: RlA (dashed line, open circles), Rl+Mxd+Dr (dashed line, closed circles), Rl+Mxd (dashed line, dotted circles), RtA (solid line, open circles), Rt+Mxd+Dr (solid line closed circles) and Rt+Mxd (solid line, dotted circles)

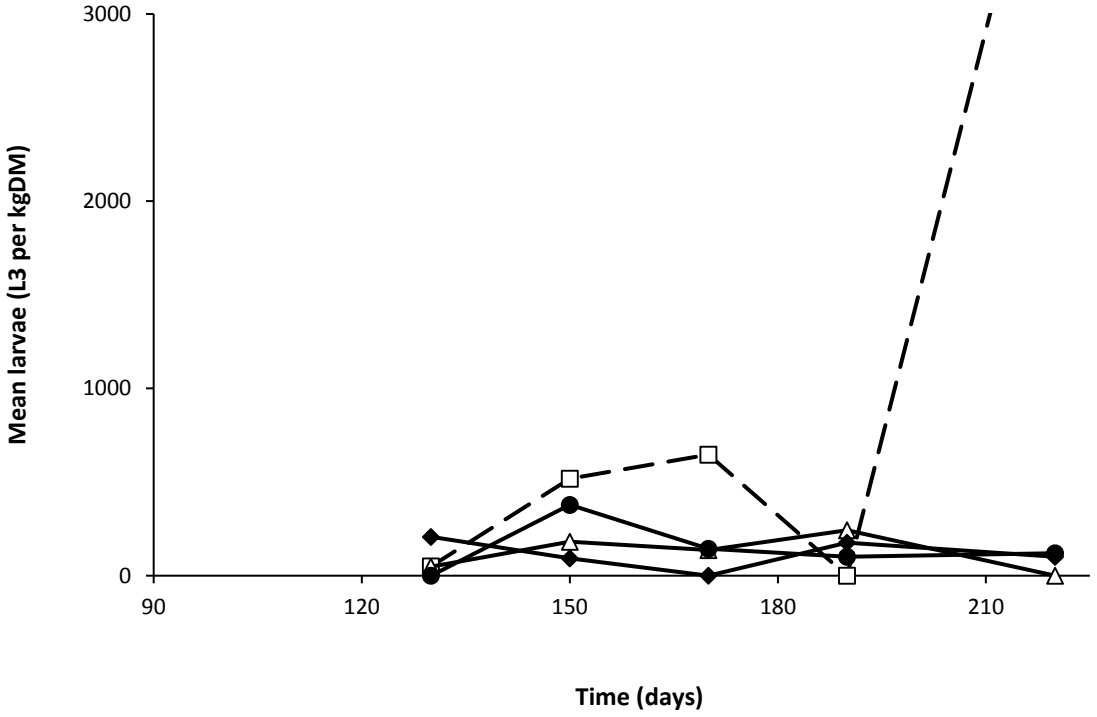
4.3.3 Pasture Larval counts

Strongyle larvae (L3 per kgDM herbage)

Mean back-transformed *Strongyle* larvae (L3 per kgDM) are given in Figures 4.5a and 4.5b for season 1 and 2, respectively. In season 1, Pasture larvae showed a tendency for treatment by time interaction (P=0.090) which reflected low initial levels that increased in RlA farmlet but remained low for the other three treatment groups

For season 2, RlA and Mxd treatment groups had greater mean *Strongyle* larvae 952.0 and 897.7 L3 per kgDM with peaks of compared with RtA (140.4 L3 per kgDM) or Mxd+Dr (78.2 L3 per kgDM) treatment groups respectively (p=0.028). There was an effect of time (p=0.050) reflecting a rise in larvae to in RlA and Mxd groups to reach peaks of 2757.5 and 1801.1 L3 per kgDM on day 210 whereas apart from a small peak in RtA and Mxd+Dr groups remained low, although this was not reflected in an interaction between treatment and time (P=0.447).

(a)



(b)

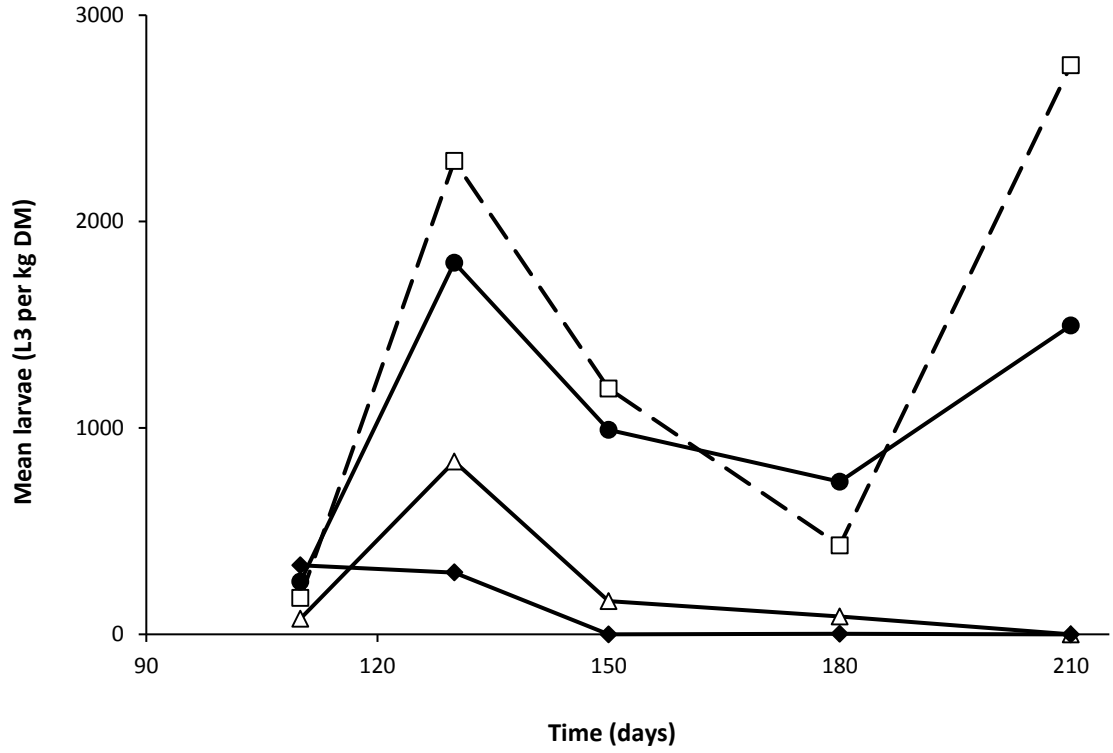


Figure 4:5 Mean back-transformed *Strongyle* infective larvae for (a) season 1 and (b) season 2 in farmlets grazed by RIA (dashed line, open squares), RtA (solid line, open triangles), Mxd+Dr (solid line, closed rhombus) and Mxd (solid line, closed circles) Romney lambs under natural mixed-species parasite infections

Nematodirus Spp. larvae (L3 per kgDM)

Mean back-transformed *Nematodirus* Spp. larvae (L3 per kgDM herbage) are presented in Figures 4.6a and 4.6b for season 1 and 2, respectively. Overall, in season 1 *Nematodirus* pasture larvae tended to increase with time (P=0.059) but there was no interaction between treatment and time (P=0.137). Generally, farmlets grazed by RlA lambs had greater mean back-transformed larvae (739.5 L3 per kgDM) compared with RtA, Mxd+Dr and Mxd treatment groups *viz.* 99.5, 141.0 and 347.7 L3 per kgDM), respectively, (P=0.052).

For season 2, *Nematodirus* pasture larvae showed an interaction between grazing treatment and time (P<0.007) reflecting a rise in larvae in farmlets grazed by RlA and Mxd lambs with peaks on day 150 and 210 while those grazed by RtA and the Mxd+Dr remained.

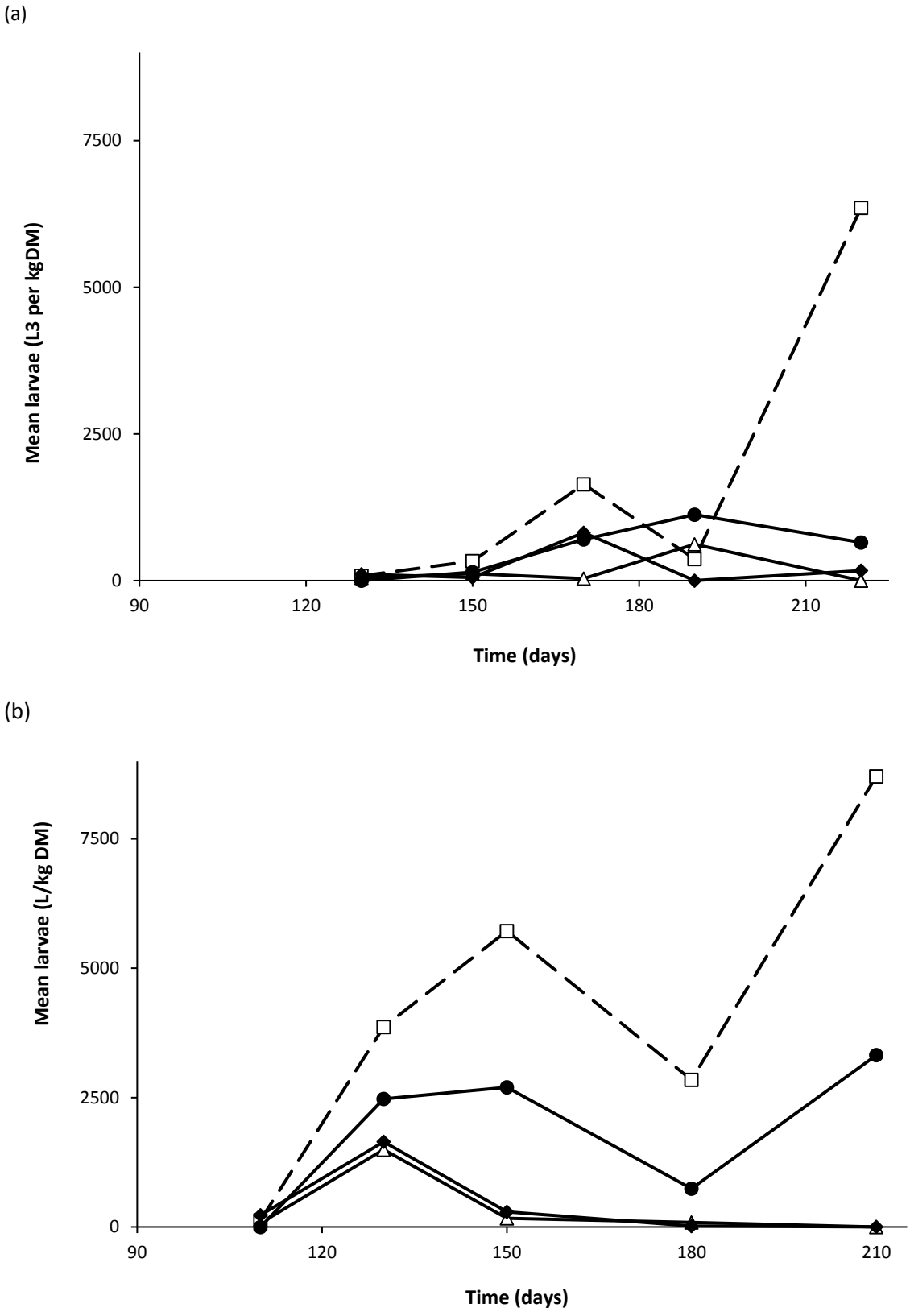


Figure 4:6 Mean back-transformed *Nematodirus* Spp. infective larvae for (a) season 1 and (b) season 2 in farmlets grazed by RlA (dashed, open squares), RtA (solid line, open triangles), Mxd+Dr (solid line, closed rhombus) and Mxd (solid line, closed circles) Romney lambs under natural mixed-species GIN parasite infections

Larvae population dynamics within farmlets for 2016-2017 season

Mean proportion of larvae population in each treatment group that were *T. colubriformis, T. circumcincta and Nematodirus* are shown in Figures 4.7a, b, c and d for RtA, RlA, Mxd and Mxd+Dr respectively. Within the different treatment groups, the occurrence of three major nematode species appeared to vary. In farmlets grazed by RtA, *Trichostrongylus* and *Nematodirus* were prevalent at the beginning but later in the study, *Nematodirus Spp*. and *T. circumcincta* species dominated. For RlA farmlets, *T. circumcincta* and *Nematodirus Spp.* were more prevalent earlier in the season but *Nematodirus* was dominant in the later stage. In Mxd farmlets, the proportions of all the three major nematode parasites species with similar *viz.* 36.5%, 32.9% and 25.0% for *Nematodirus* Spp., *T. colubriformis* and *T. circumcincta* respectively. In the Mxd+Dr treatment group, there were more *Strongyle* species earlier and *Nematodirus* later, although numbers of total larvae were low.

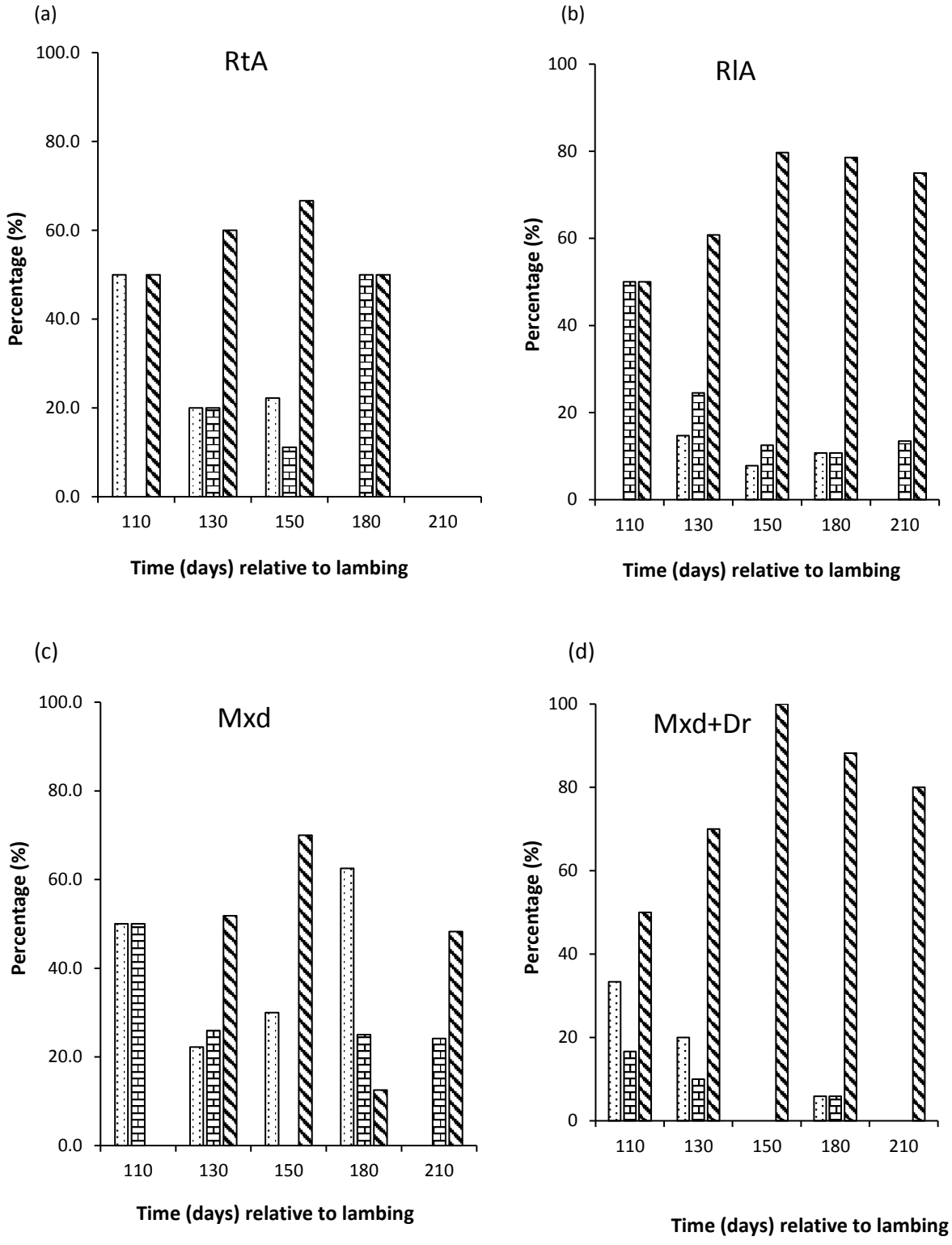


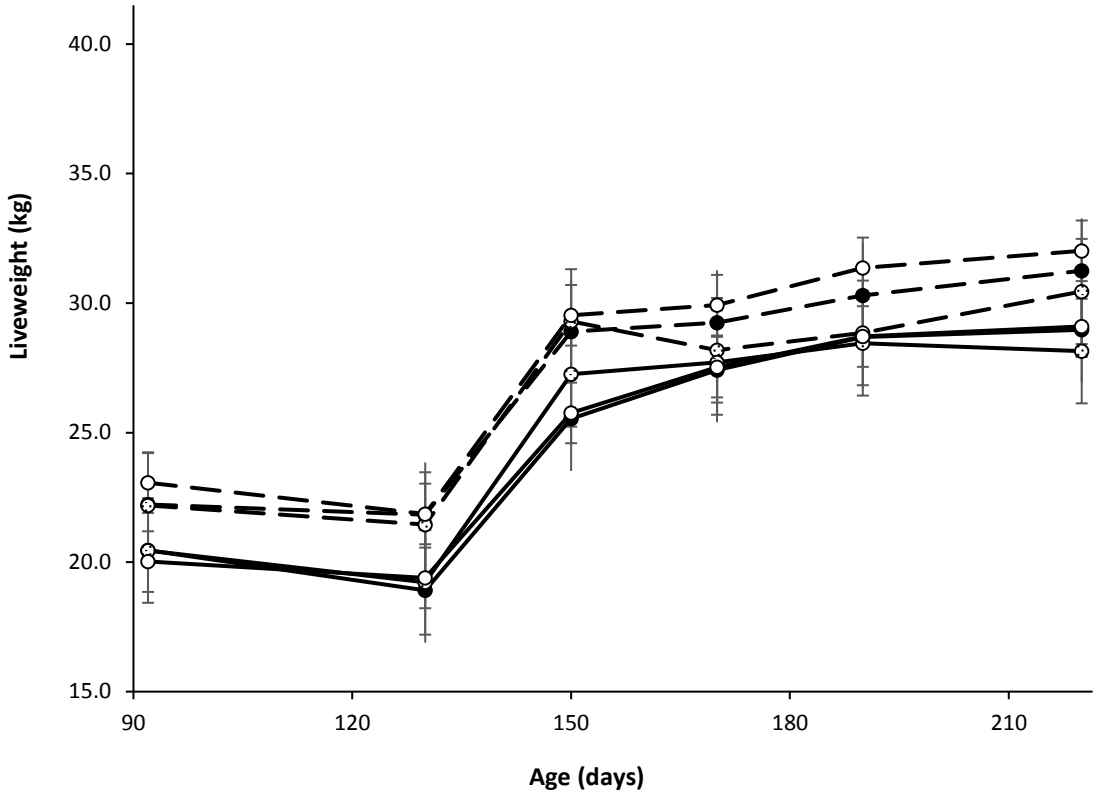
Figure 4:7 Percentage (%) of three major nematode parasite species in the different farmlets under grazing by Romney sheep lines: *Trichostrongylus colubriformis* (dotted columns), *Teladorsagia circumcincta* (brick columns) and *Nematodirus* Spp. (diagonal lined columns): a) RtA, b) RlA, c) Mxd and d) Mxd+Dr groups, respectively

4.3.4 Live weight (LW)

Mean live weight (kg) for the farmlet treatment groups is given in Figures 4.8a and 4.8b for season 1 and 2, respectively. In 2015-2016 (season 1), there was no effect of treatment on mean live weight ($P=0.465$). However, mean LW increased with time in all treatment groups ($P<0.001$) and there was no interaction between treatment and time ($P=0.347$).

For season 2(2016-2017), mean LW (kg) showed a treatment by time interaction ($P<0.001$) reflecting similar initial weights which increased with time in all treatment groups but the increase in weight was greater in Rl+Mxd+Dr and lest for the RlA treatment group, from day 150 onwards.

(a)



(b)

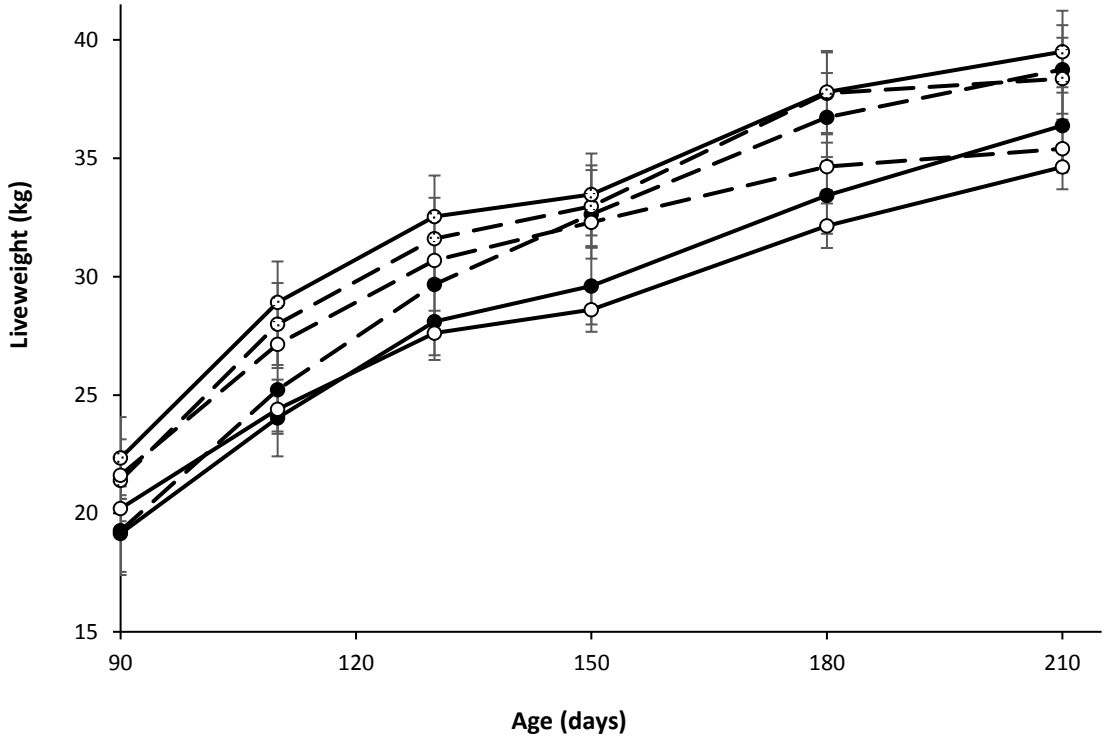


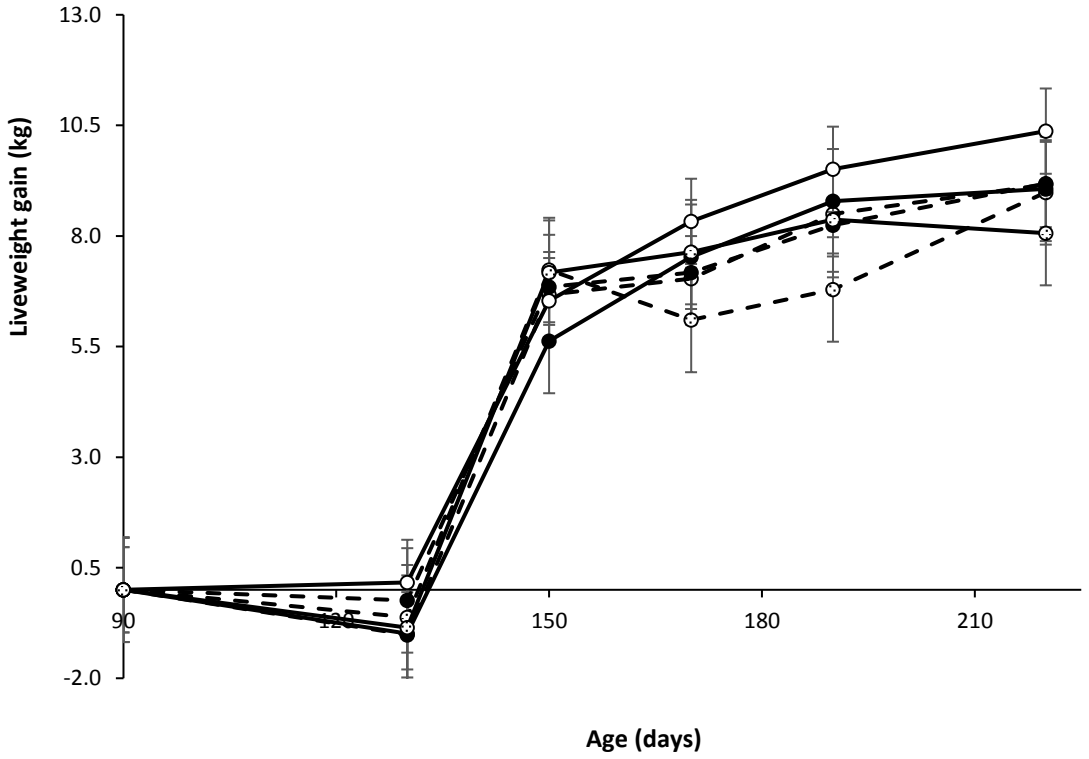
Figure 4:8 Mean live weight (kg) in Romney lambs under natural mixed-species parasite infections, (a) season 1 and (b) season 2: RlA (dashed line, open circles), Rl+Mxd+Dr (dashed line, closed circles), Rl+Mxd (dashed line, dotted circles), RtA (solid line, open circles), Rt+Mxd+Dr (solid line closed circles), and Rt+Mxd (solid line, dotted circles) respectively

4.3.5 Liveweight gain (LWG)

Mean cumulative liveweight gain (kg) of farmlet treatment groups are given in Figures 4.9a and 4.9b for seasons 1 and 2, respectively. There was an interaction between treatment and time (P<0.03) reflecting similar low initial values which increased with time in all groups but was less in Rl+Mxd between days 160 and 200.

For season 2, overall, liveweight gain showed a treatment by time interaction (P<0.001) which reflected an increase for all groups but at reduced rate in both the RlA and RtA treatments from 150 and 130 day-of age, respectively. At the end of the study period, cumulative LWG was greater in Rl+Mxd+Dr group (19.48 ± 0.91 kg) compared with Rt+Mxd+Dr treatment group (17.22 ± 0.78 kg) respectively (P<0.05, was similar between Rt+Mxd and Rl+Mxd groups *viz.* 17.16 ± 0.84 kg *c.f.* 16.99 ± 0.84 kg respectively (P>0.05) which was also similar between RlA and RtA *viz.* 14.42 ± 0.45 kg *c.f*. 13.85 ± 0.48 kg, respectively, (P>0.05) although this was temporary. However, during the first 60 days post weaning, the RlA treatment lambs had greater LW gained (178.8.1 ± 9.3 g/day) compared with RtA counterparts (144.5 ± 5.1 g/day) respectively (P<0.001). But in the last 50 days of the study, RtA lambs had greater daily liveweight gain (114.9 ± 8.6 g/day) compared with RlA counterparts (62.8 ± 14.0 g/day) respectively (P=0.002).

(a)



(b)

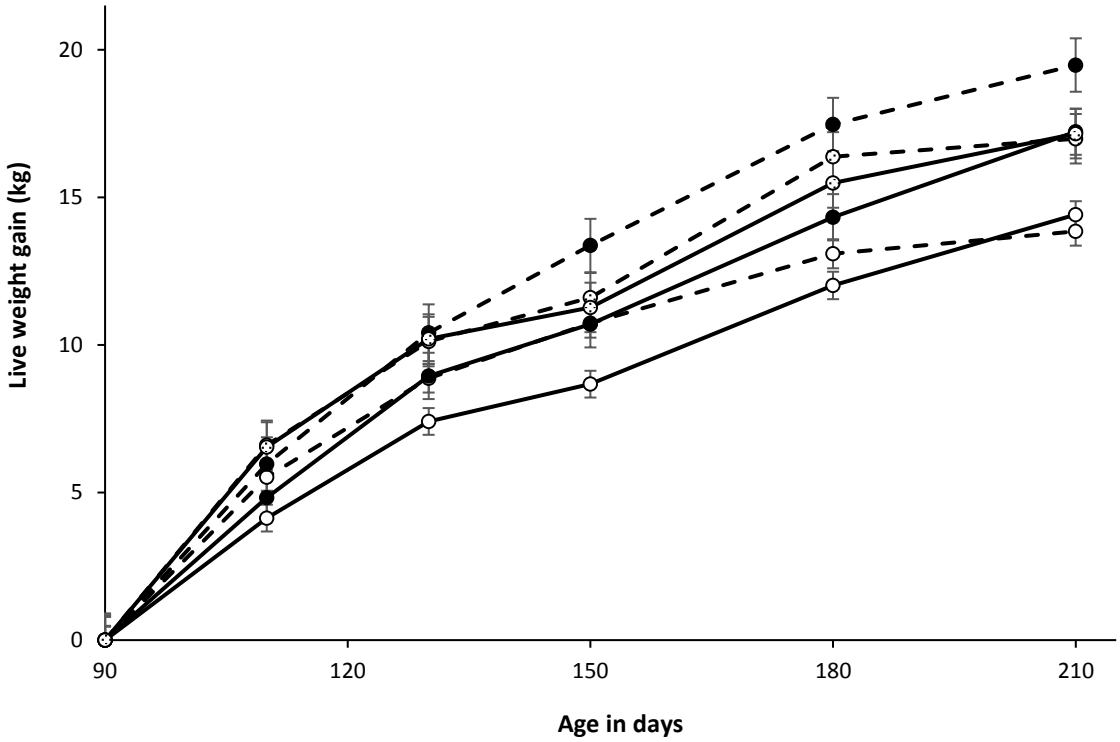


Figure 4:9 Mean cumulative liveweight gain (kg) in Romney lambs under natural mixed-species parasite infections, (a) season 1 and (b) season 2: RIA (dashed line, open circles), Rl+Mxd+Dr (dashed line, closed circles), Rl+Mxd (dashed line, dotted circles), RtA (solid line, open circles), Rt+Mxd+Dr (solid line closed circles), and Rt+Mxd (solid line, dotted circles) respectively

4.3.6 Immunoglobulin responses

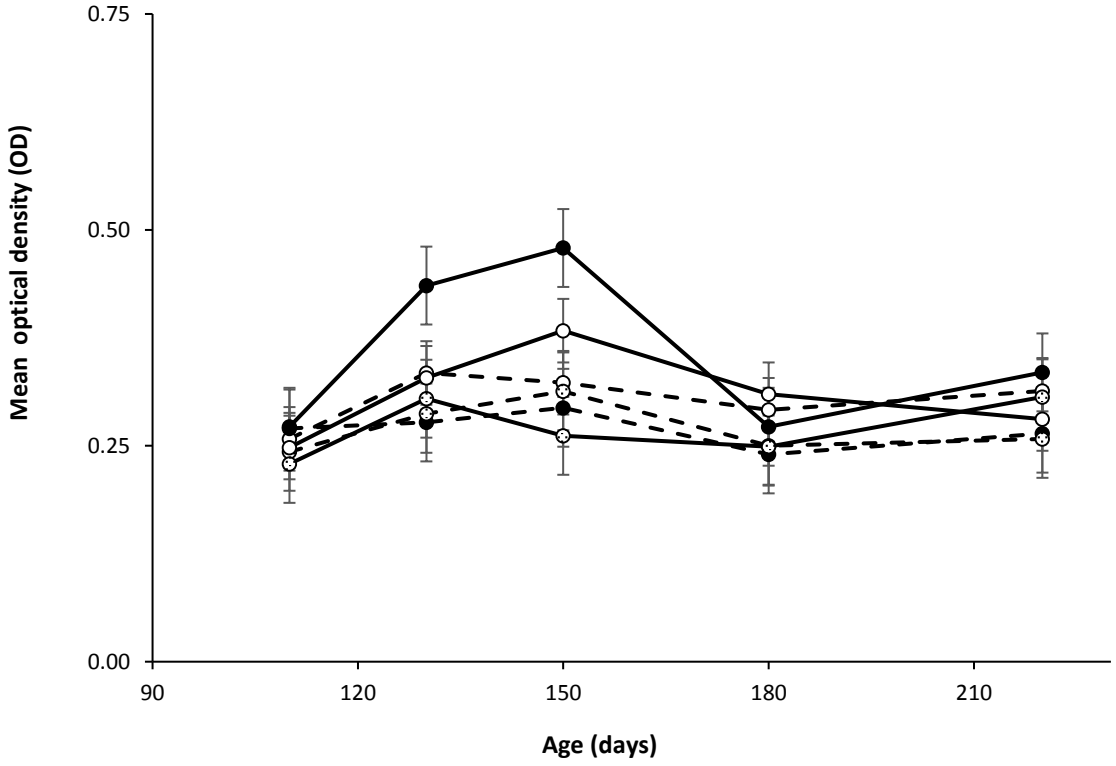
Immunoglobulin g (IgG)

Strongyle IgG

Mean *T. colubriformis* L3-specific IgG for Romney selection lines within treatment groups is given in Figures 4.10a and 4.10b for season 1 and 2, respectively. Overall, there was an interaction between treatment and time (P=0.030) which reflected a greater increase in IgG values in the RtA and Rl+Mxd+Dr groups up to day 150 but remained relatively low in all other groups

For season 2, IgG profiles showed an interaction between treatment and time (P<0.001) reflecting a low initial OD values which increased with time at greater rates in RtA and Rt+Mxd compared with RlA and Rl+Mxd while the Rt+Dr and Rl+Dr groups remained relatively low. *Nematodirus* L5 IgG profiles were similar to those described for *T. colubriformis* L3 IgG as shown in Figure 4.11 and was only performed for season 2.

(a)



(b)

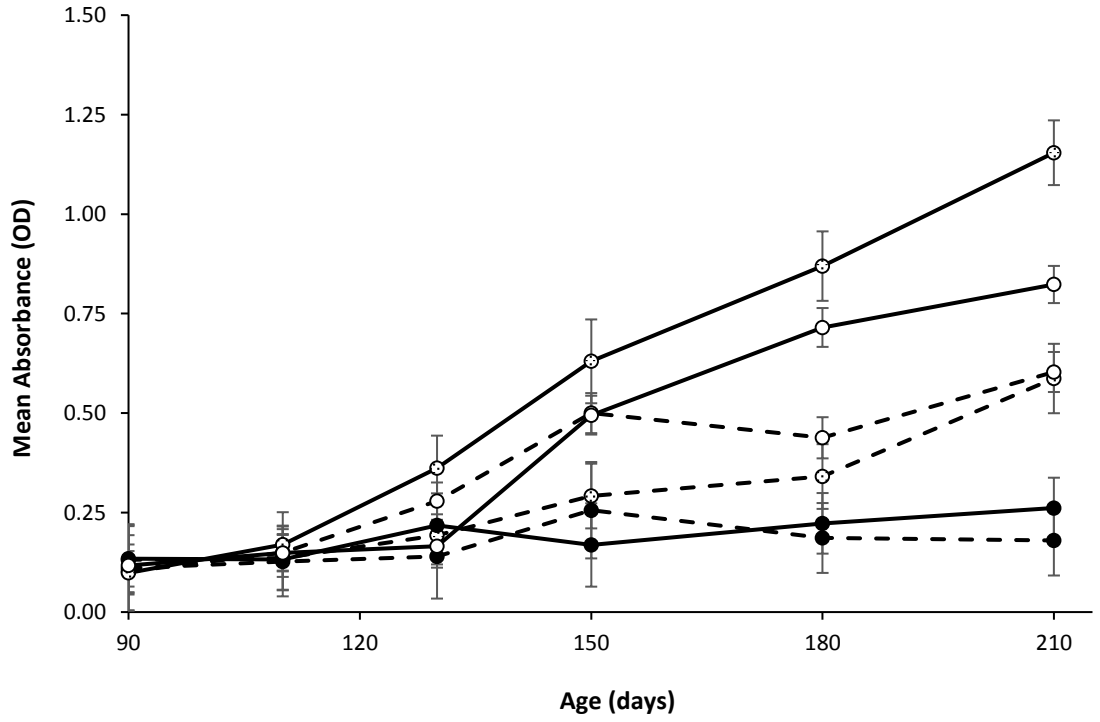


Figure 4:10 Mean optical density (OD) for *T. colubriformis* L3-specific IgG responses in Romney lambs under natural mixed-species parasite infections for (a) season 1 and (b) season 2: RlA (dashed line, open circles), Rl+Mxd+Dr (dashed line, closed circles), Rl+Mxd (dashed line, dotted circles), RtA (solid line, open circles), Rt+Mxd+Dr (solid line closed circles), and Rt+Mxd (solid line, dotted circles) respectively

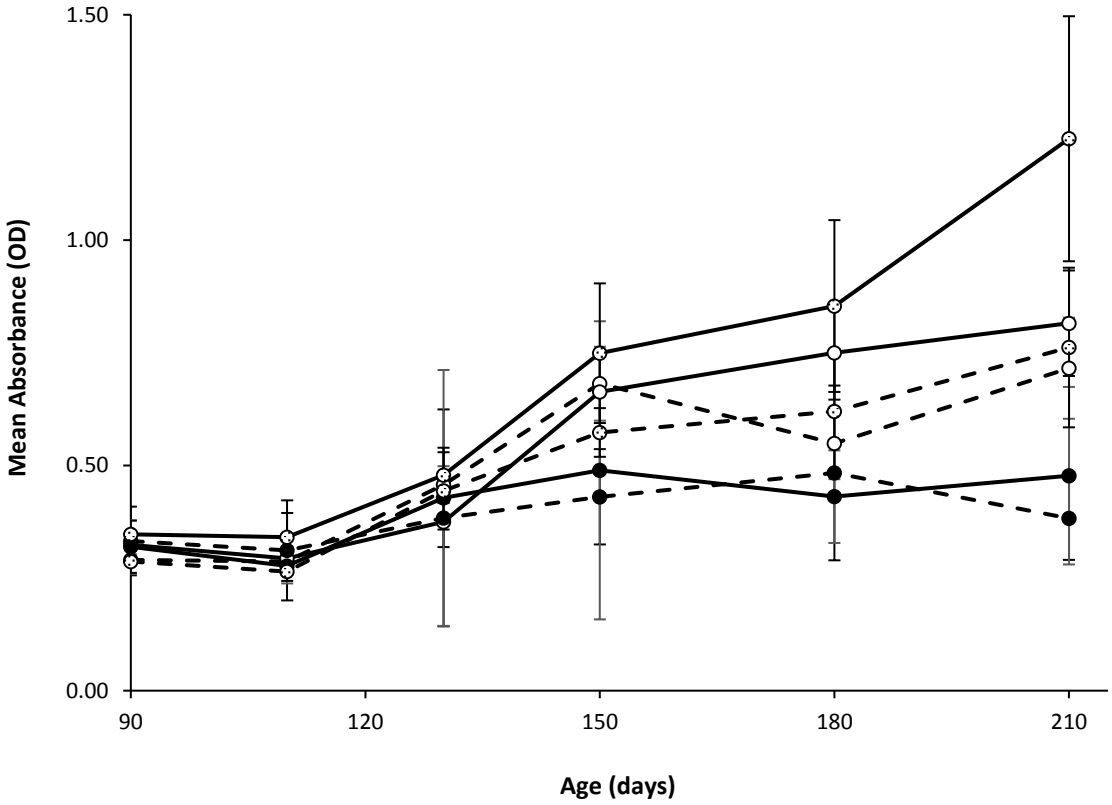


Figure 4:11 Mean optical density (OD) for *Nematodirus* Spp. L5-specific IgG responses in Romney lambs under natural mixed-species parasite infections for season 2: RlA (dashed line, open circles), Rl+Mxd+Dr (dashed line, closed circles), Rl+Mxd (dashed line, dotted circles), RtA (solid line, open circles), Rt+Mxd+Dr (solid line closed circles), and Rt+Mxd (solid line, dotted circles) respectively

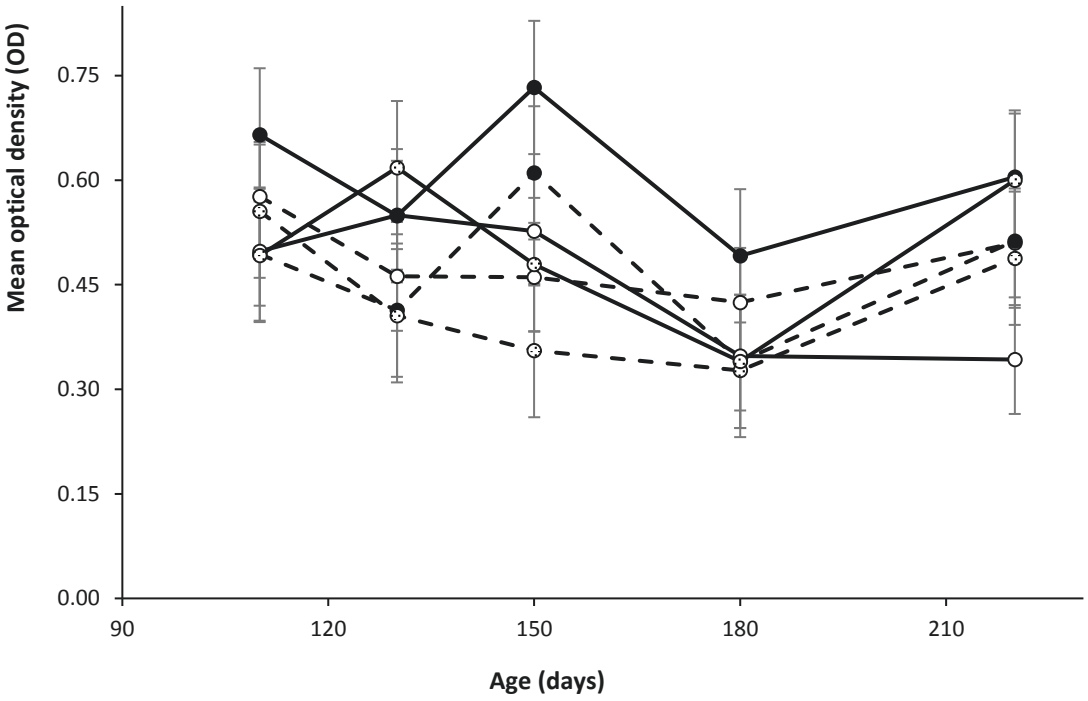
Immunoglobulin IgA

Strongyle IgA

Mean *T. colubriformis* L3-specific IgA for Romney selection lines within treatment groups are given in Figures 4.12a and 4.12b for season 1 and 2, respectively. Overall, IgA varied with time ($P<0.001$) reflecting moderately high and similar initial OD values for all groups which variable throughout the study. There were no effects of treatment ($P=0.298$) and no interaction between treatment and time ($P=0.269$).

For season 2, there was an interaction between treatment and time ($P<0.002$) reflecting consistently low values in Rl+Dr and Rt+Dr treatments whereas IgA increased with time in the other four groups but at a faster rate in Rt+Mxd compared with RtA, RlA and Rl+Mxd groups. The trends in *Nematodirus* Spp. L5-specific IgA profiles were similar to those described above for *T. colubriformis* L3-specific IgA and are given in Figure 4.13

(a)



(b)

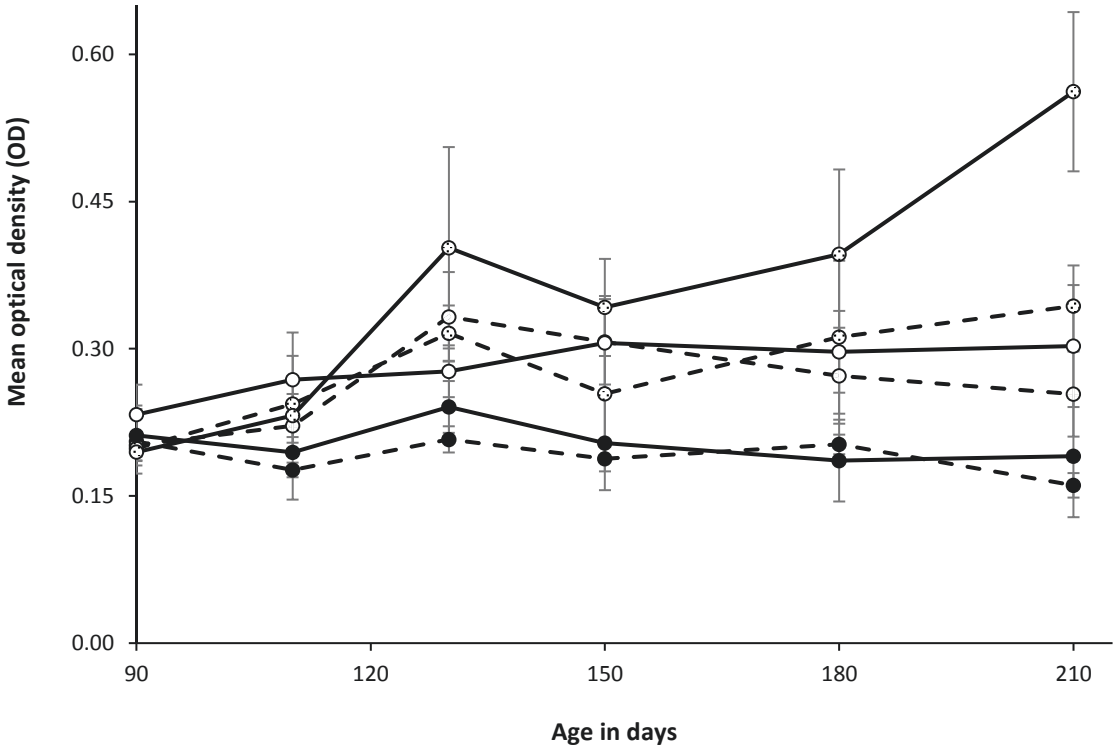


Figure 4:12 Mean optical density (OD) for *Strongyle* L3-specific antibody IgA responses in Romney lambs under natural mixed-species parasite infections, (a) season 1 and (b) season 2: RlA (dashed line, open circles), Rl+Mxd+Dr (dashed line, closed circles), Rl+Mxd (dashed line, dotted circles), RtA (solid line, open circles), Rt+Mxd+Dr (solid line closed circles), and Rt+Mxd (solid line, dotted circles) respectively

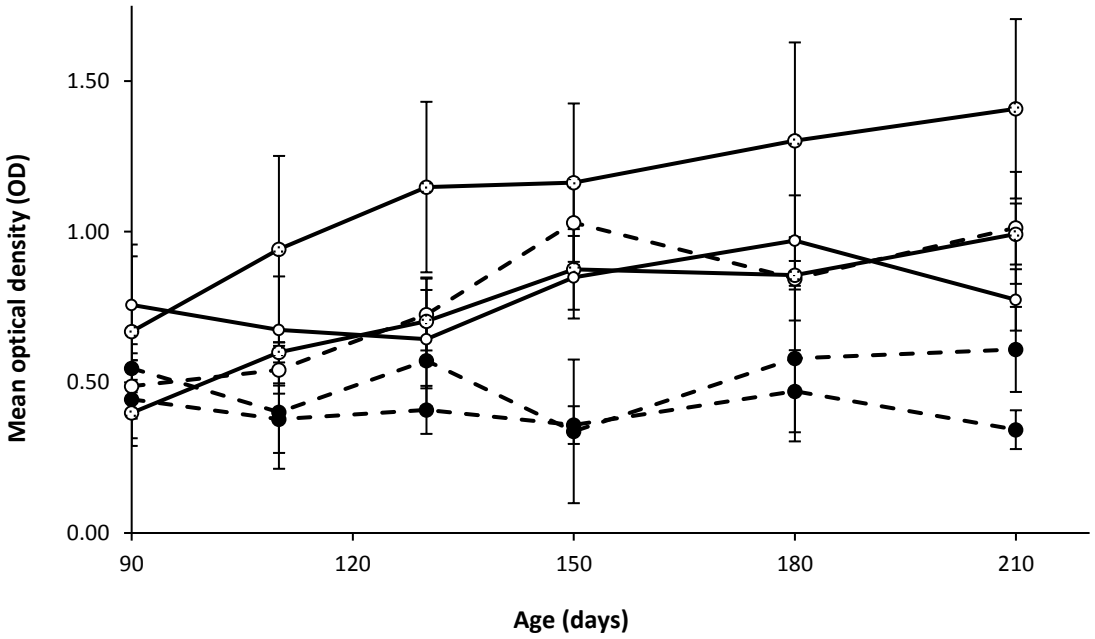


Figure 4:13 Mean optical density (OD) for *Nematodirus* spp. L5-specific antibody IgA responses in Romney lambs under natural mixed-species parasite infections (season 2): RlA (dashed line, open circles), Rl+Mxd+Dr (dashed line, closed circles), Rl+Mxd (dashed line, dotted circles), RtA (solid line, open circles), Rt+Mxd+Dr (solid line closed circles), and Rt+Mxd (solid line, dotted circles) respectively

4.3.7 Dag scores

Mean dag scores for Romney selection lines within treatment groups are shown in Figure 4.14 for season 2, There was interaction between selection line by time (P<0.001) reflecting a general increase with time until day 150 before declining. In addition, there was an interaction between treatment and time (P<0.001) which reflected an increase in dag score in resistant mixed un-drenched, resistant separately grazed and resilient separately grazed groups up to day 150 followed by a decline while the drenched groups remained low except for day 210 when there was an apparent rise in dag score in these groups. However, there was no interaction between selection line and treatment (P=0.306).

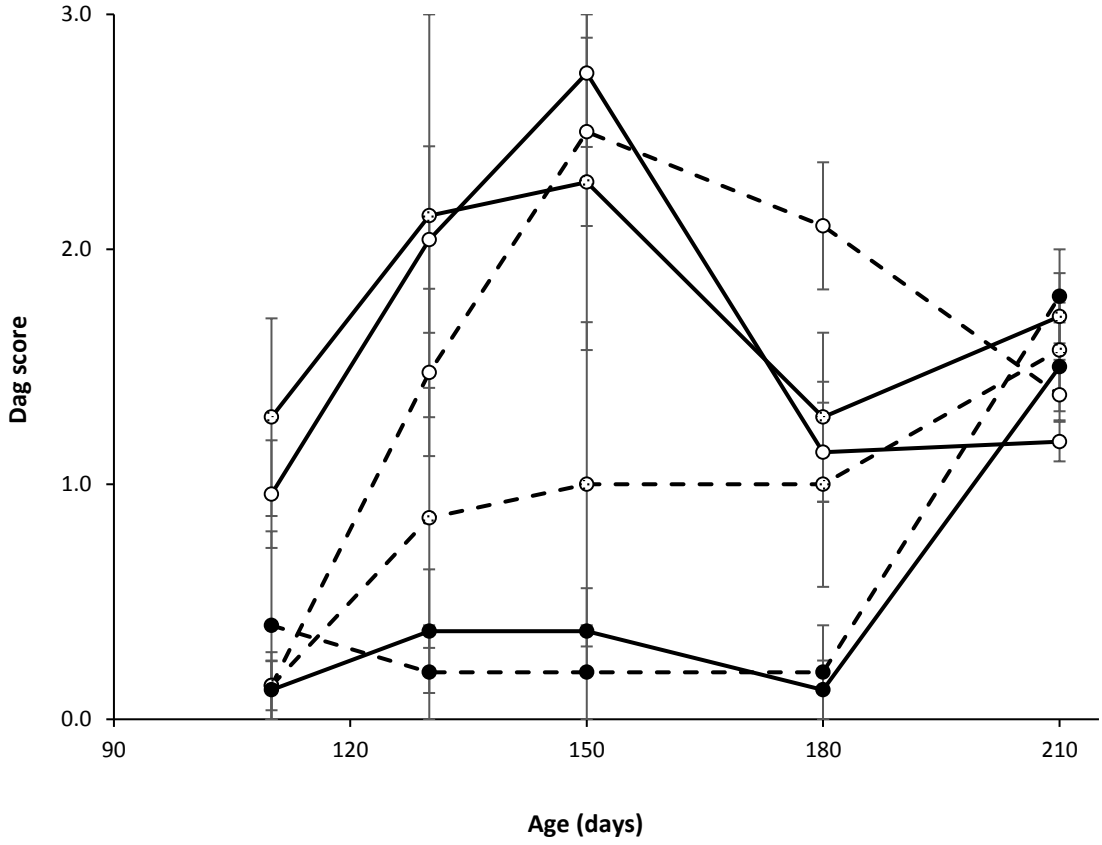


Figure 4:14 Mean dag scores for Romney lambs under natural mixed-species GIN parasite (season 2): RlA (dashed line, open circles), Rl+Mxd+Dr (dashed line, closed circles), Rl+Mxd (dashed line, dotted circles), RtA (solid line, open circles), Rt+Mxd+Dr (solid line closed circles), and Rt+Mxd (solid line, dotted circles) respectively

4.4 Discussion

4.4.1 Epidemiology

There was clear epidemiological advantage in the resistance selection line to nematode parasites in Romney sheep. Farmlets grazed by RtA treatment group maintained consistently lower pasture larvae contamination which were similar to the Mxd+Dr treatment group and largely negligible mostly being a few hundred larvae per kg DM. In contrast, there were greater (7-fold) infective larvae on pasture for both *Strongyle* and *Nematodirus* species in RlA treatment group which presumably reflects greater contamination as indicated by the high FEC in these lambs. Although larval build-up took longer in season 1, which may have largely been due to the delayed allocation of lambs into the farmlets a result of protracted pasture establishment, the observed differences in epidemiology between farmlets were consistent in both seasons. These results are consistent with previous reports where Bisset et al. (1997a) found the susceptible (high-FEC) Romney lambs had 5-6 fold greater pasture contamination compared with the resistant (low-FEC) lambs. In the current study, numbers of *Nematodirus* larvae were more than double those recorded for *Strongyle* species combined which suggests the overall economic importance of this species in sheep farming industry in the Canterbury region and presumably the entire Southern Island of New Zealand. Surprisingly, the *Nematodirus* eggs counts were much lower than those of *Strongyle* despite considerable numbers of L3 on pasture. This may suggest that either most *Strongyle* eggs did not hatch or their resulting larvae did not reach the third stage or immunity to *Nematodirus* developed earlier.

Despite the presence of greater peak pasture larvae in farmlets grazed by RlA and or Rl+Mxd treatment groups at the end of each season, there was little evidence for a considerable potential carryover of larvae that survived through the winter depicted by very low numbers at the start of the second season. Presumably, some contamination would have been provided by the contribution of initial low FEC and resulting larvae from adult ewes in season 2. Both the minimal residual larvae and larvae that might have developed from the low egg output from adult ewes could have a combined effect on the observed steady and early build-up of larvae in the second season and may help explain the greater differences between seasons. This suggestion is consistent with observation by Vlassoff (1976) who reported that lambs acquire *Trichostrongylus* larvae from a combination of residual larvae and larvae that develop from eggs deposited by ewes during the post-partum period and latter from eggs passed by lambs in autumn. However, Familton and McAnulty (1997) reported that parasite eggs deposited by adult ewes during winter form a significant contribution to subsequent infection in lambs which contrasts with observations in the current study and that of Leathwick et al. (2011) which suggest contribution from the ewes is minimal. The difference in interpretations could reflect in the underestimations based on egg numbers only when effects of faecal volume between adult ewes/cows versus lambs/calf is not included (Familton & McAnulty, 1997; Stromberg &

Averbeck, 1999; Stromberg, 1997). Unfortunately, due to factors outside the control of this study, the difference between years in the present of ewes between seasons makes direct comparisons difficult. Overall, the current study has confirmed the commonly agreed suggestion that selection for resistance to GIN parasites offers considerable epidemiological benefit in reducing pasture contamination with prospects of healthier animals in subsequent grazing.

There were variations in both magnitude of peak larval and population dynamics of the larval species on pasture among the treatment groups, particularly for the second season. Two substantial peaks in larvae in farmlets grazed by RlA group were observed with one occurring early January and the other in end March whereas for the RtA farmlet, there was one but relatively small peak early in January but remained low or negligible throughout the study period. Previous reports have documented the build-up of infective larvae on pasture with Vlassoff et al. (2001) reporting that the pattern of L3 consisted of two peaks; a small peak in spring/early summer a large peak in late summer/early autumn. The results from the current study confirms the commonly agreed suggestion that the degree of resistance to nematode parasite infection in lambs has substantial impacts on the build-up of larval on pasture as predicted from prediction models (Bishop & Stear, 2003; Dobson et al., 1990a; Laurenson et al., 2012; Leathwick et al., 1992) as well as from some field studies (Bisset et al., 1997a; Leathwick et al., 1998; Miller et al., 1998). Further, there were observable differences in the nematode parasite population dynamics between farmlets. *Nematodirus* was the dominant parasite across all farmlets and most particularly in RlA and the Mxd treatment groups. However, numbers of *Nematodirus* larvae were considerably reduced in the Mxd group relative to the RlA suggesting a benefit of inclusion of animals from resistant selection line although no such effect was observed in *Strongyle*s species. The reason(s) for such unexpected difference is not known and was outside the scope of this study, but it appears worthy of further investigation. These results are consistent with the findings of Miller et al. (1998) who reported that 3-year study, *H. contortus* was the predominant species in Suffolk lambs whereas *Trichostrongylus* spp. were abundant in resistant Gulf Coast Native lambs when they were grazed separately suggesting a greater capacity of the Native lambs to control infection with the more pathogenic *H. contortus,* although parasite species differed. Despite the short-term nature of the current study, these observations appear to suggest that selection for resistance or resilience in Romney sheep may eventually shift the parasite population dynamics if continuously grazed in isolation of each other and this may help to understanding the relative impacts of each species and the potential adaptation mechanisms.

There was a striking observation of a seemingly protracted hatching of *Nematodirus* nematode species. *Nematodirus* larvae on pasture continued to rise with time against a declining FEC from this species most particularly observed in RlA treatment group. Various authors have previously documented the importance of this nematode parasite species in the South Island regions of

Canterbury, Otago and Southland of New zealand (Brunsdon, 1967; Vlassoff et al., 2001). The protracted hatching suggests *Nematodirus* eggs survive for longer time with hatching taking place after specific temperature requirements in the range of 11-17^{o}C are met (Morgan, 2008; van Dijk & Morgan, 2008, 2009) although this was in reference to *Nematodirus battus*. The authors reported that 13^{o}C was the optimum temperature which maximized both hatching and hatching rate, and when temperature rose or fell outside the range, egg hatching stopped almost immediately. The current study took place during the summer and early autumn when temperatures were much higher than the required 13^{o}C and this may in part explain the lower *Nematodirus* pasture larvae earlier in the study despite a relatively higher FEC. Shifting from the hot summer to somewhat warm to cool temperatures in autumn combined with cold irrigation water, may have favoured egg hatch. While the study did not categorise species of *Nematodirus*, it would still be expected to be dominated by the two major classes namely *N. spathinger* and *N. filicollis* with the later known to have delayed egg hatching (Brunsdon, 1963). By implication, much of the production losses (live weight and liveweight gain) observed from such infections could be expected to have come from *N. filicollis species* although both are important.

In summary, these results have demonstrated clear evidence that selecting for nematode resistance in sheep, does provide considerable epidemiological benefits in reducing larval contamination on pasture a consequence of both the removal of existing larvae through ingestion and the lower numbers of nematodes eggs excreted. This has been achieved in the complete absence of anthelmintics, and so represents the extreme end of what may be expected.

4.4.2 Animal performance

Differences in growth potential between resilient and resistant Romney selection lines exist. The suppressively drenched treatment group was purposefully included in the design to mimic the presumed 'absence' of the effects of parasitism to understand production potential between these selection lines. From the perspective, Rl+Mxd+Dr lambs showed both greater mean progressive live weights and overall cumulative liveweight gain at the end of the study period compared with Rt+Mxd+Dr counterparts suggesting inherent differences in the growth potential in the absence of parasite challenge. These results were expected and consistent with the aim of selection for resilience which is intended to optimise productivity under reasonable anthelmintic drenching (Bisset et al., 2001; Woolaston & Baker, 1996) where, although nematodes may still be available, their population is either kept in check and or weakened to the extent they may not cause major production losses. However, the poorer performance in RtA animals has previously been attributed to the costs of immunity. It is apparent here that in addition, a difference in the growth potential may also exist.

The greater inherent genetic potential in animals from the resilient selection line may also explain the commonly reported growth advantages of these animals even in conditions when grazed together with resistant animals under infections (Bisset et al., 1996a; Morris et al., 2000; Morris et al., 1997). The existence of differences in growth in the current study where both quantity and quality of pasture may not have been optimum, suggests that these differences could even have been greater given adequate nutrients in the pasture and the opportunity to animals to express more of their growth potential.

When faced with parasite challenge with no anthelmintic treatment, animals from these selection lines i.e. RlA and RtA had both similar final weights and cumulative live weight gains which were also lower compared with their respective drenched groups, although the effects were temporal. These results were in contrast with previous reports by (Bisset et al., 1997a) which found resistant Romney lambs had greater growth rate compared with their susceptible (high FEC) Romney lambs over autumn-winter period and in both the two years when grazed separately in farmlets with minimal anthelmintic treatments to all groups. Presumably, the differences in findings may reflect a difference in the time point when such assessments were made. It was evident from the current study that in the early phase (60 days post-weaning), both LW and LWG were greater in RlA treatment group compared with RtA group which may have reflected the greater inherent growth potential of animals from the resilient line at a time when indications of infection (FEC) and larvae on pasture were moderately low. However, later in the study (last 50 days), the rate of daily weight gain in RlA group decreased suggesting increased stress from the presumed greater parasite burdens and their associated costs were impacting on the nutrient resources allocation. The current results are consistent with (Morris et al., 2010) who reported that resilient ewe lambs under low-chemical treatment struggled to maintain live-weights in autumn (March) when levels of nematode challenge were highest, but in that study all lambs grazed together and were exposed to similar parasite challenge. This prompted the authors to conclude that lambs selected for high growth under mild levels of parasite challenge (resulting from an intensive anthelmintic treatment) do not have the genetic ability to perform well when faced with much higher levels of challenge. Interestingly, both RlA and Rl+Mxd in the current study appeared to show indications of increased stress from the effects of greater levels of infection when compared with their Rl+Mxd+Dr counterparts. However, they differed on the timing when the production traits started to plateau, being a month earlier (February) in RlA compared with March for Rl+Mxd lambs. While several factors, such as level of nutrition, could contribute to this reduction in productivity, the timing also coincided with the peak larvae availability on pasture suggestive that parasitism challenge and / or loading had some contribution. Much of this difference in timing of depression may, in part, be explained by the variations in the magnitude of *Nematodirus* larvae which was consistently double in RlA compared

with Rl+Mxd treatment but both groups had similar levels of *Strongyle* larvae. The seemingly increased rate of growth in RtA observed in the last phase may suggest their potential to recoup the nutritient investments for a greater immune response to parasites earlier in their growth phase which also may in part explain the reduced rate of growth up untill day 150. Contrary, while the RtA lambs appeared to have a lower cumulative live weight gain compared with Rt+Mxd+Dr lambs, the similarity in LWG between Rt+Mxd group and Rt+Mxd+Dr counterparts was unexpected especially bearing in mind their exposure to an elevated level of parasite challenge. The similarity may possibly reflect greater weaning weights or the greater nutrtional quality in the group relative to their drenched counterparts. Overall, these results have demonstrated a clear inherent genetic difference in the growth potential between animals from Romney selection lines when grazed separately with little or no parasite challenge, being greater in RlA compared with RtA animals. However, in the presence of challenge, the production differences between the RlA and RtA treatment groups disappear with both showing similar LW and LWG.

Parasitism induced differing levels of production losses between selection lines. Lambs from the RlA treatment incurred moderate costs on production (28.9%) relative to the Rl+Mxd+Dr group whereas RtA counterparts only lost 16.3% of cumulative live weight gain of the Rt+Mxd+Dr group. The timing of this reduced rate of live weight gain in RlA group, coincided with greater levels of larvae challenge with peak of about 12,000 L3 per kg DM (in year 2) comprising mostly *Nematodirus* species and a corresponding rise in immunoglobulin G. Although the presence of lavae on pasture does not necessarily equate to worm burdens in animals (Fox et al., 2013; Litherland & Deighton, 2008), the presence of high numbers of worm eggs in lambs from this group was however indcative of the presence of greater worms numbers which, combined with antibody IgG rise, suggested animals were under increased stress from the parasite infection. Similar observations were seem in the Rl+Mxd treatment group but in these lambs the depression occurred a month later relative to RlA group, which may reflect differences in levels of challenge as well as predominant nematode species. Coop et al. (1982) demonstrated the reduction in liveweight gain in Blackface x Border Leicester lambs with directly proportional to dose rates, being 10%, 25%, and 47% in lambs infected with 1,000, 3,000 and 5,000 *Ostertagia circumcincta* larvae per day. Apart from production losses fron parasite infection and its infetcion-rate dependence, immunological responses have been reported to account for 75% of overall costs of infections (3.1kg) in Suffolk X Border Leicester/Merino lambs when administered 2000 or 4000 *T. colubriformis* L3 larvae weekly for 12 weeks (Dever et al., 2016) and 71% of this cost, occurred during the early phase coincinding with immune acquisition. Similarly, the minimal loss in productivity inccurred by RtA lambs despite a low apparent challenge may suggest that much of this may have come from self-inflicted immuonopathologies asscoiated with greater antibody IgG responses (Graham et al., 2005; Greer, 2008; Williams, 2011).

However, indoor studies by Greer et al. (2018), observed similar levels of both immune responses and indicator of intestinal pathologies between resistant and resilient-line animals when equally challenged with *T. colubriformis* L3 reflecting similar costs of infection and they suggested that differences in performance is unlikely to be attributable to the greater endogenous nutrient loss. Although RtA lambs showed some indications for the ability to recoup the nutritional investments, seen as increased growth rate later in the study, there was little evidence to suggest that they would have regained all of the lost productivity observed in season 2. Earlier, Coop et al. (1982) showed that only a proportion of the lost productivity in parasitized animals was recovered even after drenching when faced with a continued challenge. While the resilient lambs had a growth advantage over resistant lambs early in the study when infections were still low to moderate, the similarities in liveweight gains at the end may suggest the epidemiological benefits of resistance does not confer associated growth advantage at least in the short term but may in the medium to long-term when infection levels increase in RlA animals.

Parasitism is known to induce an increase in nutritional demand (Hoste et al., 2016; Poppi et al., 1990; Sykes & Coop, 1977). The higher levels of parasitism in resilient lambs combined with an apparent lower quantity of feed which had gradually declined (in all farmlets) over the study period may have exerted greater stress on resilient lambs particularly with associated costs on immune development (Greer, 2008; Hoste et al., 2016) which may explain the greater reductions in the rate of growth. However, it remains to be established what this loss in productivity translates economically. In Britain, (Nieuwhof & Bishop, 2005) found that GIN parasites were the most costly disease for sheep with 75% of the costs associated with growth reduction, which was linearly related to the severity of the infection. Whereas in Australia, Sackett et al. (2006) reported 87% loss of production was associated with nematode parasites. Clearly, reducing the impacts of the disease would have proportionally greater benefits for the producers and sheep industry in general. These results appear to demonstrate that the epidemiological advantage conferred by resistance when grazed separately, reduces the impact of nematode infection on productivity resulting in similar levels of production with resilient animals, when no anthelmintic drench is administered. But selection for resistance to GIN parasites did not demonstrate apparent growth advantage over resilient selection line, at least in the short term which suggests that the epidemiological advantage over the time frame of this study equalled the costs. The timing of when depressions in live weights or loss in liveweight gain in both RlA and Rl+Mxd lambs is important in the context of commercial farming systems where animals are raised for specific purposes. Prior to this depression, most lambs (nearly 60%) had reached the arbitrary 35 kg slaughter weight which means they may have been sent for slaughter to reduce feed costs and maximise overall farm profitability (Brown, 1990; McIvor & Aspin, 2001; Rendel et al., 2013; Thompson et al., 2016).

Contrary, animals selected for resistance to parasites may be more appropriate for replacement stock on the farm as such animals would considerably reduce the infectivity levels on pasture for subsequent grazing and reduce reliance on anthelmintic treatment for management of nematode infections.

Chapter 5

Faecal avoidance in Romney sheep lines selected for resistance or resilience to gastro-intestinal nematodes

5.1 Introduction

Grazing animals make decisions around faecal avoidance based on existing parasite burden and immune status, thus reducing the risks of further parasite infection (Fox et al., 2013; Hutchings et al., 2001). Pasture swards around faeces are often highly contaminated with infective L3 larvae (Hutchings et al., 2007) and as ruminants cannot detect L3, the presence of faeces has been suggested as a deterrent for the potential detrimental health risk associated with particular sward patches (Cooper et al., 2000; Hutchings et al., 1998; Hutchings et al., 1999). Contamination of pasture swards with faeces initiates grazing avoidance due to smell (Aoyama et al., 1994; Dohi et al., 1991) and this odour could be more pronounced in faeces from parasitised animals due to increased leakages of nitrogenous compounds from the damaged digestive tissues (Bown et al., 1991; Poppi et al., 1986). A combination of the sensation of discomfort of ingesting contaminated pasture, visual cues of presence of faeces and height or colour of forage appear to play an important role in enforcing grazing decisions by ruminant animals. Further, the extent of faecal avoidance has been shown to be influenced by both the previous exposure of animals to parasites and also the host immune status, with parasite-naïve and immune animals exhibiting lesser faecal avoidance than their parasitized counterparts (Hutchings et al., 1998; Hutchings et al., 1999). Sheep with an indication of higher levels of parasite infection showed a greater avoidance of faecal swards than animals with lower infection rates (Hutchings et al., 2002). A study by Seó et al. (2015) also reported that susceptible cattle with higher levels of parasitism grazed further away from faecal contaminated patches compared with resistant counterparts. While faecal ('parasite') avoidance behaviour would be beneficial to host animals with very limited ability to mount an effective immune response to assist with (Fox et al., 2013), such animals run the risks of potential failure to ingest enough nutrients which, if sustained, could potentially result in a nutritional disadvantage.

In lines of Romney lambs that have undergone selection for resistance, resilience or susceptibility to parasite challenge (Bisset et al., 1996a; Bisset et al., 1994; Bisset et al., 1996b; Morris et al., 2010), differences in levels of infection have been reported when grazed on pasture compared with indoor fixed challenges. In field challenges, where animals from these lines are grazed together and are left to selectively graze, resistant animals are able to maintain very low faecal-egg counts (FEC), seldom above 200 eggs per gram of faeces (epg; Chapter 3) while the faecal-egg count of resilient-line

animals is consistently in excess of 1000 epg. This difference has been attributed to the greater immune capacity of resistant-line animals which limits parasite establishment and/or fecundity compared with animals selected for resilience to nematode infection (Douch et al., 1995; Douch et al., 1994; Shaw et al., 1999). However, when animals from Romney breeding lines selected for either resistance or resilience were raised indoors and given a constant and equal parasite challenge of 2000 *Trichostrongylus colubriformis* L3 larvae per day, FEC profiles between the lines were more similar, with peak FEC of 1200 and 1700 epg for resistant and resilient lines, respectively (Greer et al., 2018). This difference in response when given a constant parasite challenge then raises the possibility that the much lower FEC observed in resistant animals when grazing pasture may, at least in part, be a result of greater faecal aversion which reduces their exposure to infective larvae. From this perspective, Perendale sheep from lines selected for resistance have been reported to be less willing to graze pastures which are heavily contaminated with infective larvae (Hutchings et al., 2007) which may, in turn, contribute to their lower infection levels. Therefore, the objective of this study was to determine if a difference in faecal avoidance exists between lines of Romney sheep selected for resistance or resilience.

5.2 Materials and methods

5.2.1 Animal Measurement

Aversion to either a mud-clay or faecal ball was assessed in Romney lambs from lines that had undergone selection for either resistance or resilience to parasite challenge. Animals were part of a larger cohort that were maintained in pasture-based farmlets where resistant and resilient lines grazed separately and were consequently exposed to differing levels of parasite challenge. On four occasions, at a mean of 130, 150, 180 and 210 days-of-age, which spanned the anticipated periods of immune development to gastro-intestinal nematodes, 10 resistant and 10 resilient lambs were removed from their farmlet and housed in individual pens. Animals were fasted overnight with access to fresh water. Each animal was then presented with two swardlets (measuring 39 cm x 29 cm each) in succession, consisting of newly-sown ryegrass that had never been grazed to graze for one hour at the end of wich animals were then returned to their original farmlets until the next assessment time.

At each sampling time indicators of the animals parasite status and immunological status were assessed immediately prior to housing. For parasite status, faecal samples were obtained directly from the rectum of each individual for the determination of the concentration of nematode eggs per gram of faeces (faecal egg counts; FEC, epg) using a modified McMaster method with a sensitivity of 100 epg as described in Chapter 3. An indicator of immune status was determined using *Trichostronglyus cobubriformis*-speficic L3 antibodies in saliva collected using a mouth swab which was then centrifuged at 12,000 x g and the liquid collected and stored at -20 ^{o}C until analysis as described in Chapter 3.

5.2.2 Herbage mass and faecal avoidance measurements

Prior to grazing and immediately following grazing the herbage mass in each swardlet was recorded using a miniture rising plate meter which had been calibrated using herbage cuts from the same pasture which was then dried in an oven at 70^{o}C until a contstant weight was obtained. Using a string template each swardlett was divided into 24 section (6 x 4), the size of each matched the plate meter area. The height of the plate for each section was then recorded and the herbage mass prior to and post-grazing for each section calculated.

At the centre of one swardlet a faecal ball made from compressed freshly produced faeces measuring approximately 8 cm in diameter and weighing 50g was placed and on the other a mud-clay ball of the same dimension and weight was placed at the center. Animals were allowed access to each swardlett for one hour. The order of presentation of either faecal ball or mudclay ball was randomly decided for each animal by the flip of a coin.

Each animal was presented with two swardlets (measuring 39 cm x 29 cm each) in succession consisting of newly-sown ryegrass that had never been grazed. Faecal avoidance (cm) was determined using a measurement of distance from the faecal or mud-clay ball that was grazed. Following the presentation of the second swardlett the animals were then returned to their original farmlets until the next assessment time. The swardlets were trimmed to ground level after each testing time. A summary of the events at each sampling time are given in Figure 5.1

Figure 5:1 Cycle of events during entire experimental time

5.2.3 ELISA

Immunoglobulin G (IgG) antibody specific to *T. colubriformis* L3 in saliva was measured using an ELISA as described by (Greer et al., 2018) and summarised in Chapter 3. Briefly, 100 ul of neat saliva was incubated in duplicate in 96 well plates that had been coated with 0.1 µg *T. colubriformis* L3 antigen per well. Following washing with PBS (pH 7.2 + 0.05% Tween 20), each well was incubated with 100 µl of horseradish peroxide (HRP) conjugated polyclonal rabbit anti-sheep IgG immunoglobulins (Pierce Immunopure Antibodies, cat #31480, lot #GI959969) at a dilution of 1:4,000. Colour was developed using 100 µl of 0.05 M phosphate-citrate buffer adjusted to pH 5 with 0.02% of 30% H2O2 added and containing 100 µg of Tetramethylbenzidine dihydrochloride (TMB; Sigma Aldrich, U.S.A) for 25 min before the reaction was stopped with the addition of 1.25 M $H2SO_4$. Colour intensity was measured using a microplate reader (Multiscan Go, 1510-01462C, Thermofisher Scientific, Finland) at 450 nm. Results were expressed as the mean optical density (OD) value of the duplicate wells and adjusted according to a standard positive serum sample present on each plate.

5.2.4 Statistics

Saliva IgG and faecal egg count data were analysed using GENSTAT statistical package (version 18.1.0.17005, 2015, VSN International Ltd, U.K.) with genetic line and time as factors after undergoing sequential comparison of ante-dependence structures for repeated measures and then analysed by Restricted Maximum Likelihood (REML) with animal included as a random effect. Herbage mass offered was compared using a general analysis of variance (ANOVA) with genotype, treatment (mud-clay or faecal ball) and time as factors. Faecal avoidance at each time was assessed using a paired T-test using the pairing of grazing distance for each animal when offered swardlets containing either a mud-clay or faecal ball. Data from one resilient animal was excluded from the analysis because it failed to graze for all the 4 testing times. Two other resilient animals did not consistently graze, one on three occasions and one on two occasions. Data from these animals was excluded from the analysis for faecal avoidance and herbage consume but were included in the repeated measures analysis for IgG and FEC.

5.3 Results

5.3.1 Parasitological profiles

Back transformed mean FEC (epg) are given in Fig. 5.2. Overall, there was an effect of selection line (P<0.001) but not time (P=0.206) and there was no significant interaction between selection line and time (P=0.175) which reflected consistently greater FEC in resilient compared with resistant animals.

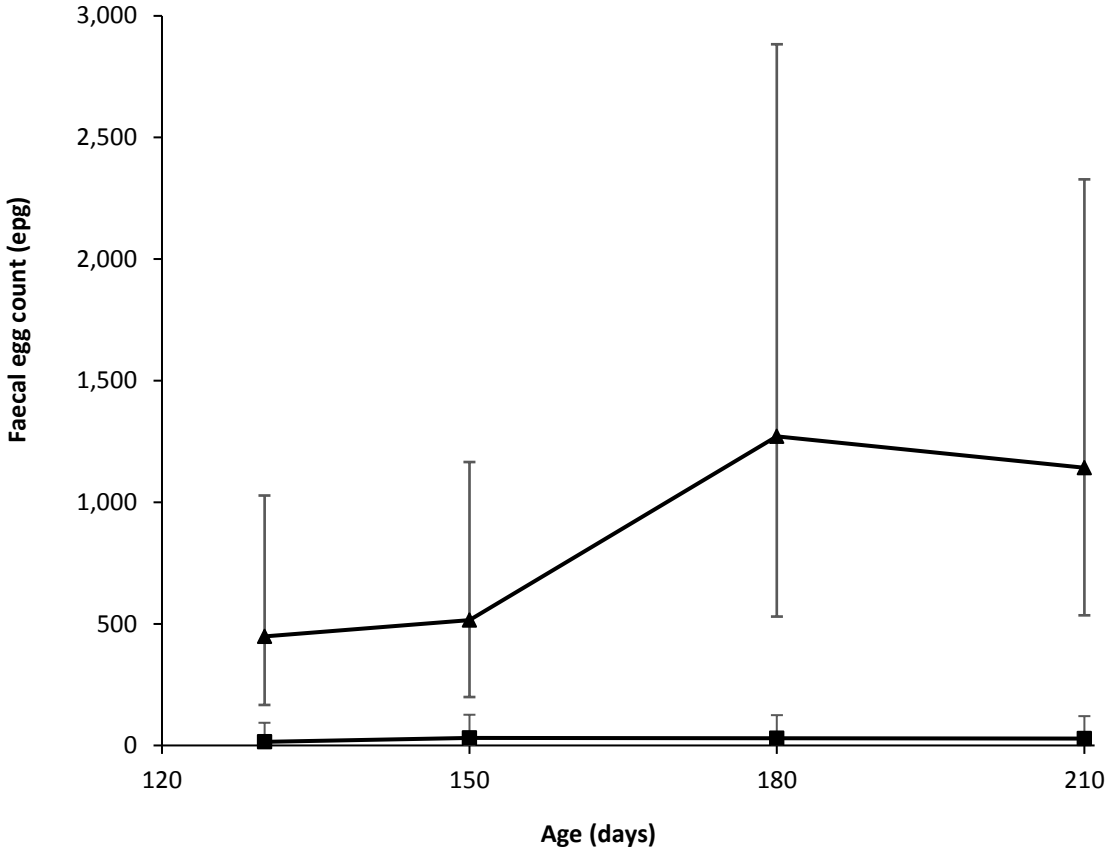


Figure 5:2 Mean back-transformed faecal egg count (± 95% confidence interval) at each sampling time for Romney lambs from lines selected and maintained in their own farmlets for resistance (squares) or resilience (triangles) to gastro-intestinal nematodes

5.3.2 Immunological profiles

Immunoglobulin IgG

Mean optical densities (OD) for L3 *T. colubriformis*-specifc immunoglobulin G (IgG) are given in Figure 5.3. Overall, IgG was greater in resistant than in resilient animals (P=0.005) and this increased with time (P<0.001) but there was no interaction between selection line and time (P=0.130).

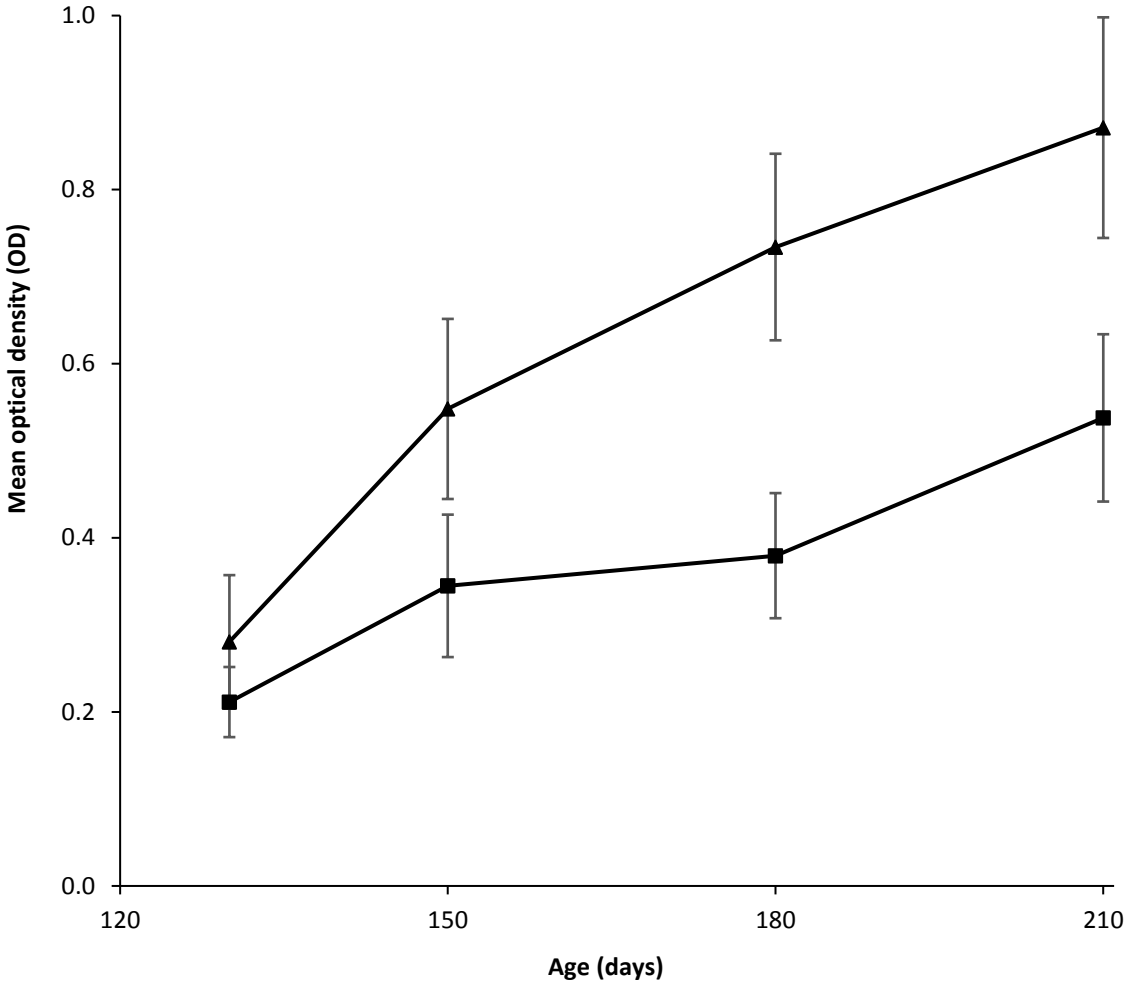


Figure 5:3 Mean (± s.e.m) optical density (OD) for *Trichostrongylus colubriformis* L3 -specific IgG antibody for Romney lambs from lines selected for resistance (triangles) or resilience (squares) to gastro-intestinal nematodes at each sampling time

5.3.3 Herbage mass (g)

Mean herbage dry matter offered to animals reduced with time (P< 0.001) but was similar between lines at each point; day 130, *viz.* (24.75 c.f. 25.55 g), day 150, *viz.* (20.78 c.f. 19.67 g), day 180, *viz.* (20.32 c.f. 20.17 g), and day 210, *viz.* (14.72 c.f. 14.96 g) for resilient and resistant, respectively. Overall, mean herbage dry matter consumed decreased with time (P<0.001) but was similar for both resistant and resilient animals, *viz.* 7.60 ± 0.43 g and 7.03 ± 0.50 g, respectively, (P=0.300).

5.3.4 Grazing distance (cm)

Mean distance grazed to either a faecal or mud-clay ball is given in Figure 5.5. Overall, animals grazed further away from the faecal ball, *viz*. 11.53 ± 1.138 cm compared with mud-clay ball, *viz*. 4.19 ± 0.995 cm (P<0.001) and there was a tendency for resilient-line animals to graze a greater distance from objects, *viz*. 8.63 ± 1.14 cm compared with resistant-line animals, *viz.* 7.09 ± 0.99 cm (P=0.093). The grazing distance decreased with time (P<0.001) but there were no interactions between selection line and type of object (P=0.709) or selection line with time (P=0.738).

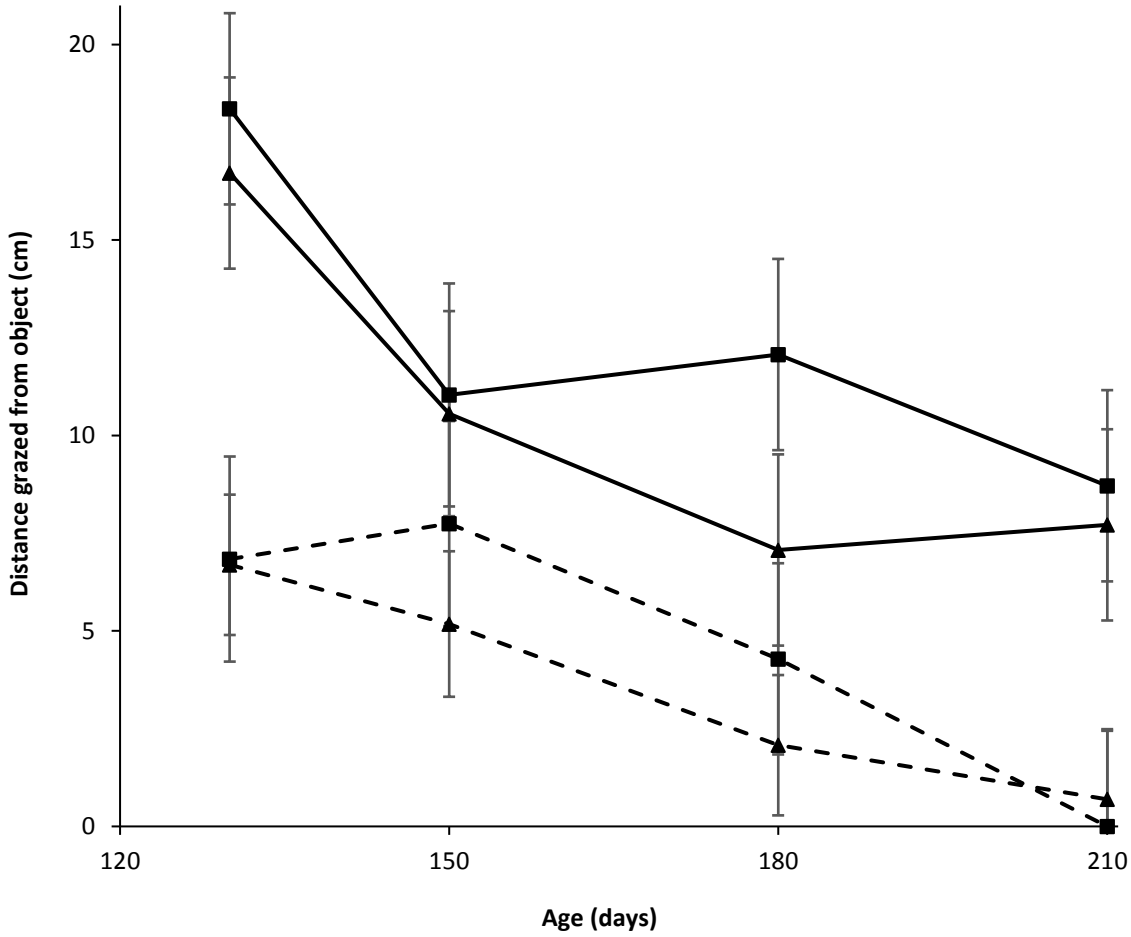


Figure 5:4 Mean (± s.e.m.) grazing distance from either a faecal ball (solid line) or mud-clay ball (dashed line) when offered in swardlets for Romney lambs at different ages from lines selected for resistance (triangles) or resilience (squares) to gastro-intestinal nematodes

Figure 5:5 Pictorial view of grazing distance from faecal ball (top row) and mud-clay ball (bottom row)

5.4 Discussion

A clear difference in phenotypic indicators of parasite infection existed between the selection lines. Resistant-line lambs had FEC that were consistently below 100 eggs per gram (epg) of faeces, whereas their resilient counterparts had a peak FEC in excess of 1000 epg. Despite the fact that animals may have been exposed to different levels of challenge on their farmlets, these observations are consistent with previous reports from Romney selection lines when grazed together (Bisset et al., 1996a; Bisset et al., 1994; Bisset et al., 1996b; Morris et al., 2010). Similarly, the greater IgG antibody responses in resistant animals are in agreement with previous investigations in lines selected for resistance or susceptibility to infection (Bisset et al., 1996b; Douch et al., 1995; Douch et al., 1994; Shaw et al., 1999). Further, despite maintaining a high FEC the resilient-line animals showed evidence of immune development through increasing parasite-specific IgG antibody, which is in agreement with previous observations of animals from these lines (Greer et al., 2018). Combined, these observations give a clear indication that these animals were a fair representation of their respective selection lines and their phenotypic response to field infection is consistent with what was expected.

Both lines showed similar levels of faecal-specific grazing aversion, being consistently willing to graze closer to the mud-clay than the faecal ball as given in Figures 5.4 and 5.5. Although the presence of a mud-clay ball provides an artificial stimulus, the grazing distance from this gives an indication of the aversion an animal may have to an object *per se*. The balance of the greater grazing distance consistently recorded for the faecal ball then reflects the faeces-specific aversion, which was similar for both the resistant and resilient lines. Faeces-specific avoidance in parasitised animals has been reported previously to be affected by the animal's immune status and state of parasitism (Hutchings et al., 1998; Hutchings et al., 1999) and has been suggested to be beneficial for the animal to reduce their potential exposure to larval challenge (Fox et al., 2013). This is important in the context that a greater proportion of larvae (approximately 90%) can migrate horizontally but remains mostly within 30 cm distance from the faecal mass (Almeida et al., 2005; Familton & McAnulty, 1997; Gronvold, 1989). Despite a tendency of resilient animals to graze further away from any object, the absence of faecal-specific differences between these Romney selection lines despite their differences in infection levels and immune responses was unexpected. These results contrast with a previous report that genetically resistant Perendale sheep were less willing to graze swards (tussocks) that were heavily contaminated with parasitic larvae (Hutchings et al., 2007) and the findings of Seó et al. (2015) that cattle with high levels of parasitism had greater faecal avoidance and grazed further away from dung compared with medium to low level infection animals. Although differences exist in the design between this study and those of Hutchings et al (2007) and Seo et al (2015) in that they were conducted in the field while the current study was indoors, there was a similarity in the principle intention to assess indirectly in the case of Hutchings et al (2007) and directly (Seo et al., 2015) and

this study on how animals deferring in immune status were willing to graze close and or avoid faecal contaminated swards. The typically low FEC observed in resistant-line animals when grazing has been accepted as being due to a greater immunological capacity to reduce nematode establishment and/or fecundity (Bisset et al., 1996b; Shaw et al., 1999). However, when animals from these lines were raised indoors and given a constant and equal challenge of *T. colubriformis* L3 larvae (Greer et al., 2018), the differences between the lines in FEC were much less evident than when grazing pasture. While the reasons for the differences between grazing and indoor studies remain unclear, the results of the current study suggest that the lower FEC in resistant animals when grazing is not due to a greater faecal aversion which may reduce exposure to infective larvae.

Avoidance behaviour did appear to decrease with time. A reduction in aversion was observed with both selection lines increasingly becoming more willing to graze closer to both the faecal and mud-clay balls. Reduced faecal avoidance has been associated with increases in animals' immune status (Hutchings et al., 1999; Hutchings et al., 2001) although there is limited evidence from the current study to support this. Despite the reduction in object avoidance with time in both lines coincided with a corresponding rise in *T. colubriformis*-specific IgG levels, the consistently lower IgG profiles in resilient animals was not associated with differences in faecal-specific aversion, indicating factors other than immune development may be a primary driver of faecal avoidance. With this in mind, however, in the current study the herbage offered at each time did decline which raises the possibility the reduced grazing distance may have simply reflected the animals becoming less averse to any object in an attempt to satisfy their hunger. Alternatively, animals may have become familiar with the presence of objects which indicates some learning may have occurred. Faecal avoidance has been accepted as a learned behaviour with previous parasitological experience influencing willingness to graze close to faeces (Hutchings et al., 1998; Hutchings et al., 1999). Therefore, despite the resilient animals maintaining a considerably greater FEC, the lack of difference in faeces-specific grazing avoidance between these selection lines that did not change with time may simply reflect a lack of suffering they perceived themselves to be experiencing and the associated learning this may have afforded.

In summary, faecal-specific grazing aversion existed and was similar in magnitude for animals from both the resistant and resilient selection lines and did not appear to be directly associated with either differences in parasite loading or changes in immunological status. These results do not support the suggestion that some of the difference in parasite loading between the selection lines may be a consequence of greater faecal avoidance in resistant animals that reduces their potential exposure to infective larvae whilst grazing.

Chapter 6

General Summary, Conclusions and Future Research Prospects

Combined, these results demonstrated that considerable differences exist in both the epidemiology and production potential between lines selected for resistance or resilience to nematode infection. On the one hand, epidemiological advantage can be confirmed through grazing resistant animals alone, but on the other hand this does not result in a clear production advantage, owing to the reduced growth potential relative to resilient-line animals. Moreover, the relative advantage of selecting for either resistance or resilience was temporal, largely reflecting a difference in the timing of when each line started to react to the parasite challenge. Resilient animals until this point were able to maintain acceptable levels of performance despite greater contamination being applied. In contrast, resistant animals appeared to develop immunity and prevent the cycling of parasite eggs from very early age, which can be attributed to earlier immune recognition and development, rather than differences in grazing behaviour.

Selection for resistance exhibited a clear epidemiological advantage in reducing pasture larvae contamination by around 7-fold relative to the animals selected for resilience (Chapter 4). Resistance to nematode infection is known to be associated with reduced larvae establishment, retarded development, reduced worm fecundity and reduced worm egg viability (Dobson et al., 1990c; Kemper, 2010; Morris et al., 2000; Stear et al., 1997; Stear et al., 1995; Stear et al., 1999). Although these effects were not specifically evaluated, the combined effects may have considerably contributed to the expected lower larvae contamination on pasture in farmlets grazed by resistant-line animals alone. Despite the high anticipated variabilities in quantifying pasture larvae (Litherland & Deighton, 2008) and difficult to attain with precision, these findings are consistent with previous reports by Bisset et al., (1997) who found the high-FEC Romney lambs had 5-6 fold greater pasture contamination or infectivity compared with the resistant (low-FEC) lambs. Similarly, these results appear to confirm the computer simulation models which predicted greater epidemiological benefits of selecting for resistance (Bishop & Stear, 2003; Dobson et al., 1990a; Laurenson et al., 2012; Leathwick et al., 1992; Leathwick et al., 1998) with Laurenson et al. (2012) estimating approximately 40% and 83% reductions in FEC and pasture contamination expected by grazing resistant animals alone. The ability of resistant-line animals to reduce nematode egg excretion and by implication larvae contribution on pasture, was evident during the study both when grazed alone (Chapter 4) and / or when in combination with resilient animals (Chapter 3 and 4). As both selection line animals were shown to have similar chances of exposure to potential risk of parasite infection when grazing (Chapter 5), these results have clearly demonstrated the importance of selection for resistance to

nematode parasites and its associated epidemiological benefits in reducing pasture larvae infectivity. These results are consistent with the findings of Eady et al (2003) which reported 69% reduction in FEC and by implication pasture larvae was achieved by genetic selection for resistance. This effect of reducing pasture contamination was also evident in the mixed undrenched treatment groups where presence of animals from the resistant line resulted in a considerable reduction in larvae population of *Nematodirus* species although this was not the case for *Strongyles*. Despite the unexpected observation of the lack of effect on *Strongyles*, the reasons for which are not known, the results do point to the suggestion that over time, resistance may lead to healthier grazing environments for animals by the net removal of existing larvae whereby larvae ingestion was greater than larvae contamination.

Nematodirus spp. (but not *Nematodirus battus*) appeared to be the dominant parasite in all the treatment groups despite differences in the magnitude while *Strongyles* species varied. While *Nematodirus* was abundant in all lines, the magnitude appeared to differ between resistant and resilient groups and even in the mixed undrenched treatment (Chapter 4). In addition, the greater numbers of *Nematodirus* within resilient treatment group which was also more than double relative to those for *Strongyle* species combined, suggests that selection for resilience may concurrently be selecting for *Nematodirus* species. The increase in larvae occurred despite numbers of *Nematodirus* FEC declining over the same period suggestive of a protracted hatching ability of this species. The protracted hatching of *Nematodirus* eggs also reflects species ability to survive for longer time on pasture and synchronized hatching occurring only when specific weather related requirements are met (Morgan, 2008; van Dijk & Morgan, 2008, 2009), and may suggest that the greater larval contamination observed in second season, some contribution may have come from the eggs of previous season consistent with Familton and McAnulty (1997) that a significant amount of larvae overwinters. With regards to *Strongyles* species, grazing resistant and resilient animals separately may favour *T. colubriformis* in resistant animals whereas in resilient-line animals *T. circumcincta* appeared to be abundant early which declined with time, an observation which is consistent with the findings of Miller et al. (1998) discussed in Chapter 4. Despite the short-term nature of the current study, these observations appear to suggest that selection for resistance or resilience in Romney sheep may eventually shift the parasite population dynamics if continuously grazed in isolation of each other and this may help to understanding the adaptation strategies of parasites and in estimating the relative impacts of each species to animal welfare.

Overall, there were clear demonstrable epidemiological benefits of selecting for nematode resistance in reducing pasture contamination with infective larvae which may have been derived from both the removal of existing larvae through ingestion and the excretion of lower numbers of nematodes eggs.

The dominance of *Nematodirus,* in animals selected for resilience may help in understanding the impacts of this species in the overall cost of parasitism on production often observed in resilient animals.

There were temporal differences in the timing of development of immunity between selection lines when exposed to nematode challenge which may account for the considerable epidemiological differences. Both lines developed immunity when infected, but immunity occurred earlier in resistant compared with resilient animals regardless of whether resistant animals were grazed alone (Chapter 4) or together with resilient-line animals (Chapters 3 and 4), being 40 chronological days earlier (Chapter 3). Despite a delay in reaching the apparent immunological threshold response, resilient lambs still displayed evidence of their capacity to develop an effective immunity to infection with similar magnitude of peak parasite-specific immunoglobulins to those produced by resistant-line animals. However, the longer time taken for immunity to develop allowed for the greater larval build-up with considerable contamination of pasture from the greater nematode egg deposition prior to immune acquisition. The early activation of the adaptive immune response observed in resistant-line animals, was consistent with findings of Greer et al. (2018) which suggested that rapid immune capacity in resistant animals was independent of infection and that it may be present even in the absence of infection. Combined with the absence of differences in grazing behaviour, particularly with respect to avoidance of faecal contaminated patches and their associated high parasite risks (Chapter 5), suggests that both line animals have similar exposure to antigenic stimulus but differ in either their capacity to recognise this or the threshold levels required to stimulate immune development. Further, the presumed differences in threshold may not be related to body size. Idris et al. (2011) reported that animal body measurements and / or differences in animal frame sizes was not directly associated with parasite infection in lambs of different genotypes when experimentally infected with *H. contortus*. Nonetheless, the early presence of a greater and competent immune response in resistant-line animals may effectively have suppressed incoming larvae establishment, their development and may be expected to have reduced worm length and associated reduction in fecundity (Bisset et al., 1996b; Douch et al., 1995; Douch et al., 1994; Gill, 1991; Green et al., 1999; Kemper et al., 2010; Shaw et al., 1999) reflected in consistently low FEC and correspondingly reduced larvae on pasture. The earlier than expected immune resposes reported in studies by Greer et al. (2018) may have reflected that the animals in this indoor study were naïve before infection or that the dose and the resulting establishment of incoming larvae were higher than those encountered when grazing (Bisset et al., 1997a) particularly when lambs are born on pasture.

Given that selection pressure for resistance occurs at a set chronological age and does not take into account the physiological age differences between selection line animals, the temporal nature of immune development may have contributed to the commonly held suggestion that resilient animals

have a lesser immunological capacity to handle nematode infections. Immune development was associated with physiological maturity. At the same chronological age, animals selected for resistance appeared to be physiologically more advanced relatively to those selected for resilience (Chapter 3). Although the presence of mating marks only served as an indicator of sexual maturity, onset of puberty in resistant females occurred earlier than the resilient females and was also associated with an earlier and greater rise in cumulative proportion of resistant animals that attained an IgG threshold of 1-OD indicative of presence of effective immunity. The animal's physiological state of growth is known to be correlated with nutritional requirements and corresponding production of adipocytokines, such as leptin, from the increased accumulation of fat as carcasses mature (Ahima et al., 1997; Blache et al., 2000; Boisclair et al., 2000; Delavaud et al., 2000; Delavaud et al., 2002; Greer et al., 2009a; Lord et al., 1998), which are also associated with T-cell immune modulation (Lord et al., 1998). Animals that are relatively more advanced in maturity can be expected to express more adipocytokines signalling (Ahima et al., 1997; Ahima et al., 1996) and if this occurred, the early heightening of these adipocytokine-derived signals may have enhanced the antigenic stimulus required for adaptive immune activation in resistant animals.

Overall, the relatively short-term findings of this study showed no differences in performance between selection lines in the presence of nematode challenge when no anthelmintics were administered. Both resilient and resistant animals displayed similar final live weight and cumulative liveweight gain suggesting the epidemiological benefits of nematode resistance was equal and / or similar to the costs of infection in resilient-line animals, despite the effects being temporal. Although this was achieved in the absence of anthelmintics and represents the extreme of what may be expected, these results support the suggestion that selection for resistance is a sustainable long-term strategy for nematode parasite management (Bishop et al., 2004; Bishop & Stear, 2003) and will greatly reduce the costs associated with anthelmintics (Besier & Love, 2003; Leathwick & Besier, 2014; Pomroy, 2006; Sutherland & Leathwick, 2011) while achieving similar animal productivity as observed in the current studies (Chapters 3 and 4). Earlier on in each of the studies, however, productivity of resilient-line animals were greater than their resistant counterparts, but in the latter phases, the depression in both live weight and liveweight gain coincided with greater indications of infection (FEC), greater larvae challenge on pasture and a corresponding rise in immunoglobulins suggesting increased nutritional stress of infection and its associated costs of immune development on production (Coop & Kyriazakis, 1999; Coop & Kyriazakis, 2001; Dever et al., 2016; Greer, 2008; Greer et al., 2005). This was irrespective of whether the selection line animals were grazed separately (Chapter 4) or together (Chapter 3). Contrary, resistant animals with both greater physiological advancement in maturity and a presumed early nutritional investment in adaptive immunity, effectively suppressed nematode infection to the extent that the benefits were recouped

later in the studies when their performance continued to accelerate. Whether the performance of resistant animals would have eventually surpassed their resilient counterparts beyond the study period is not clear and difficult to extrapolate. However, these results are in agreement with the findings of Bisset et al., (1997) which reported greater productivity in resistant compared with resilient when grazed in isolation in autumn-winter period which also coincided with greater larvae in resilient animals. However, they contrast with observations in other studies with these Romney selection lines that reported greater productivity of susceptible than resistant animals when grazed together under similar nematode exposure (Morris et al., 2000; Morris et al., 1997) and even in Perendale selection lines (Morris et al 2005). The disparity in findings, may reflect the timing when such comparisons were made, as earlier highlighted, that when assessment is performed early, resilient animals display greater growth when infection levels are low to moderate while their resistant counterparts increasingly direct much of the nutritional resources towards developing an effective immune response to infections which contribute to their poorer performance during this phase. Contrary, when performance assessments are done later, resistant-line animals appear to accelerate with greater liveweight gain whereas for resilient animals, greater impacts of parasitism can be observed through depression in live weights and reduction in liveweight gain.

In the 'absence' of nematode challenge, clear differences in performance exist between the selection lines. Resilient-line animals had greater weaning weights compared with resistant counterparts implying they may have been born heavier, a direct result of selection for increased production. Not only were weaning weights greater, resilient lambs also displayed greater progressive mean live weight throughout much of the study period, which allowed a greater percentage of animals from this line to reach the slaughter weight of 35 kg at an earlier stage than resistant animals (Chapters 3 and 4) indicative of greater inherent growth potential. The greater production potential of resilient-line animals was evident in both absence of parasites (mixed drenched group, Chapter 4) and in presence on nematode challenge (Chapter 3) although temporal in the latter case. These results were consistent with greater genetic progress reported within the New Zealand sheep industry in decades of selection for improved production (Amer, 2009; Morris et al., 2010; Young & Wakelin, 2009) which on a national scale has been driven by buyers' commitment, breeders' motivation, genetic technology, animal breeding research and extension (Young & Wakelin, 2009). Further, the findings were also consistent with the goal and objectives of commercial farming systems which is aimed at increasing profitability and is dependent on optimising efficiency of farm resources to best achieve both set production targets and higher premium prices earlier in the season (McIvor and Aspin, 2001; Rendel et al 2013; Thompson et al 2016). However, striving for greater genetic improvement for increased production, has also been associated with animals that often do not perform well under high levels of parasite challenge (Bisset et al 2001; McEwan et al., 1992, 1997;

Eady et al., 1998; Morris et al., 2010), particularly in the absence of concurrent selection pressure for resistance. This was evident in the present study with resilient and resistant-line animals ending with similar live weights in separate (Chapter 4) or combined (Chapters 3 and 4) grazing in the absence of anthelmintics. Contrary, the lesser growth potential in resistant animals both in absence (Chapter 4) or presence of infections (Chapter 3 and 4) led to failure of the majority of animals from this selection line to attain a given set of slaughter weight (35 kg). Consequently, these animals may have to stay longer on-farm which may affect both resource use efficiency and the overall profitability of the farming enterprise (Brown, 1990; McIvor & Aspin, 2001; Rendel et al., 2013; Thompson et al., 2016) unless if they are to serve as replacement stock and thus have a greater opportunity to recoup the investment in immunity. Previous studies investigating the production differences between animals selected for resistance or resilience have reported greater levels of productivity in resilient animals (Morris et al., 1995, 1997, 2000, 2005, Watson et al., 1992, 1997, Woolaston, 1992). Most often these selection lines were compared when run together prompting the suggestion that this may have resulted in underestimation of the impact of host genotype on LW, LW-gain, FEC, and pasture contamination due to potential masking effects. This was one of the objectives in the study to assess the production potential of these lines when run in separate farmlets under challenge with no anthelmintic treatment as reported in earlier section and in Chapter 4.

There were, however, individuals within each selection line that did perform well while others did not (Chapter 3) which suggested that within each line, selection is possible to incorporate both immunity and growth. Nearly all of the animals in each line with <20 kg weaning weights were immune, but their growth profiles were below average suggesting that continued selection based solely on FEC as an indicator trait of resistance will continue to favour animals with poor growth performance. In contrast, animals with weaning weights >23 kg were more likely to grow well but not all were immune. Of the 29 animals with weaning weights >23 kg, 13 (representing 45%) were also immune with very low FEC whereas 55% had greater FEC and thus likely to contribute to larvae contamination on pasture. The high FEC-shedding animals exhibited lower IgG and IgA responses suggesting a general poorer immune capacity. It was apparent from the study that such animals could readily (>90%) be identified by sampling once for either IgG at about 220 day-of-age or FEC at 270 days-of age respectively. The remainders (immune and growth) being the desired animals expected to grow well with a combined enhanced nematode immune response which will maintain considerably low FEC and low consequential pasture contamination. The end product of which is highly anticipated by the commercial producers. It would be of interest to understanding the genetic variations associated with growth in these animals and how they differ from those that are immune but with below average growth. Overall, these observations are consistent with the suggestion that a combined selection for both growth and immune traits is possible (Bishop and Stear, 2003, Wheeler

et al., 2008; Bisset et al., 2001) and should be encouraged in selection programmes and commercial production.

Parasitism induced moderate costs on production in resilient animals (28.9%) but less in resistant (16.3%) relative to their respective drenched counterparts. In resilient animals, much of the impacts of parasitism was evident in the later phase of the study between February and March which also coincided with both substantially large numbers of larvae on pasture, with the majority being *Nematodirus* species (>12,000 L3 per kgDM), and the commensuration of immunological IgG responses (Chapter 4). The acquisition phase of immunity to nematode infections is nutritionally costly (Greer 2008; Sykes, 2008) from the mass proliferation of immunoglobulins which are largely comprised of proteinaceous cells, resulting in reduced nutrients resources allocation to productive process (Coop and Kyriazakis, 1999, 2001). Consistent with costs of immunity, Dever et al (2016) reported that immunological response accounted for the majority (75%) of the overall cost of infection (3.1 kg), and 71% of this cost occurred in the phase which coincided with the acquisition of immunity. All animals in the current studies were under similar plane of nutrition as reported in both Chapters 3 and 4, which means any differences in rate of liveweight gain may likely be attributed to the impact of nematode challenge and associated costs of immunity. And presumably, much of the costs associated with parasitism, may have been caused by *Nematodirus* which was the dominant parasite species. These results are consistent with the suggestion that the magnitude of the production losses depends on the rate of infection and parasite species involved (Bisset et al., 1997; Coop et al 1982; Sykes, 2008; Symon et al., 1981) besides host animals age, immune and nutritional status. While the differences in production losses in the current study (Chapter 4) reflected differences in levels of challenge between lines, recent indoor studies by Greer et al., (2018) reported similar costs of infection on performance when animals from these selection lines were equally challenged with *T. colubriformis* L3. Interestingly, when animals were grazed together with similar exposure to potential infections (Chapter 3), overall cumulative liveweight gain were also similar between resistant and resilient-line animals despite the temporal nature of the effects and differences in levels of infection. Further, there were observations that within resistant and resilient selection lines, 30.4% and 26.7% had a combination of resistance and growth, and these animals also showed an early development of immunological IgG and IgA profiles and had similar growth profiles which suggested that other factors other than costs of immunity are involved but highlighted the potential for combined selection which could reduce the relatively large production losses in observed in resilient-line animals.

In summary, the series of experiments from this study have clearly demonstrated that selection for resistance to nematode parasites has favoured animals that are lighter in body weight but physiologically more mature at the same chronological age with an early timing of immune

development which effectively controls FEC, eventually resulting in considerable epidemiological benefits.

Selection for resistance would substantially and sustainably reduce the costs and dependence on anthelmintic treatment as a means for controlling nematode parasites while achieving similar productivity. However, grazing resistant and resilient animals in isolation of each other appeared to shift the larval population dynamics with greater numbers of *Nematodirus* in the resilient animals while the inclusion of animals from the resistant selection in mixed grazing was effective in reducing *Nematodirus* species. These Romney selection lines appeared to have similar faecal / parasite avoidance grazing behaviour and are potentially exposed to similar levels of nematode parasite infections. Thus, variations in parasite loading and potential for contamination of pasture could be attributable to the differences in the timing of acquired immunity which occurs early in resistant than resilient although the mechanism and magnitude of responses based on immunological profiles appear to be similar. The clear demonstrable increased growth potential of resilient animals under anthelmintics or when faced with infections early in their lifetime, suggests they provide the best opportunity in the short-term for the commercial farming systems which aim to quickly finish and send lambs for slaughter to fetch higher premium prices. However, the presence of animals in both selection lines with both enhanced immunity and production traits, is highly indicative of the possibility for potential combined selection in breeding programmes and commercial production conditions.

Potential Future research

Morgan et al. (2018) have compiled a snapshot of 100 questions which detail current concerns and perceived priorities highly considered by researchers as important for further study in livestock helminthology. One of the compiled questions regards defining circumstances under which breeders should aim for either resistance or resilient to helminth infections. Results from the current study, particularly the greater differences in timing of immune development and associated costs on production, have provided initial insights of the implications and potential opportunities regarding selective breeding for resistance or resilience to nematode in Romney sheep. However, still more needs to be done. Pertinent questions such as whether breeding for resilience (productivity under challenge) will break down above certain thresholds needs to be investigated. Further investigations are warranted to determine the long-term impacts of epidemiological benefits of resistance on production and potential prospects of shifting the parasite population dynamics when grazed separately from resilient animals. What is also less clear is the economic impacts of reduced larvae availability from selection for resistance versus the lost and / or foregone opportunity costs for greater growth in resilient-line animals. Worth considering also is the presence of animals from both selection lines which exhibited both immunity and growth traits. Understanding the genetic composition/constitution of these animals and isolating what makes them different from animals that are immune but do not grow well under challenge, may help speed-up the progress of combined trait selection. Animal grazing behaviour is another complex but potential area that still require further attention in an attempt to understand the defence strategies used by animals under challenge to avoid larvae ingestion. While in the current study both lines appeared to exhibit similar grazing behaviour, the contrasting outcomes between these results and others from previous field studies are indicative of the many unanswered questions.

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